INTRAMEDULLARY REAMING AND NAILING OF FEMORAL SHAFT FRACTURES

A STUDY ON THE PROCEDURE-RELATED INFLAMMATORY- AND CARDIOPULMONARY RESPONSES

Thesis by

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PREFACE

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ABBREVIATIONS

a	activated (used with factors)
AIS	Abbreviated Injury Scale
ALI	Acute lung injury
ANOVA	Analysis of variance
ap	anterio posterior
ARDS	Adult respiratory distress syndrome
A_2M	$Alpha_2$-macroglobulin
BP	Blood pressure
BW	Body weight
CARS	Compensatory anti-inflammatory response
CI	Indexed cardiac output
CO	Cardiac output
CRP	C-reactive protein
CT	Computer tomography
CVP	Central venous pressure
CW	Carcass weight
EDTA	Ethylenediaminetetraacetic acid
F	Factor
FES	Fat embolism syndrome
FiO_2	Fraction of inhaled oxygen
Hb	Hemoglobin
HR	Heart rate
ICU	Intensive care unit
IEMR	Institute for Experimental Medical Research

IL	Interleukin
IMN	Intramedullary nailing
IMP	Intramedullary pressure
ISS	Injury severity score
MAP	Mean arterial pressure
MODS	Multi organ dysfunction syndrome
MOF	Multi Organ Failure
MPAP	Mean pulmonary artery pressure
NISS	New injury severity score
ns	Not significant
PA	Pulmonary artery
$PaCO_2$	Partial pressure of arterial carbon dioxide
PAI-1	Plasminogen activator inhibitor-1
PaO_2	Partial pressure of arterial oxygen
P_AO_2-P_aO_2	Alveolo-arterial oxygen difference
PCWP	Pulmonary capillary wedge pressure
PE	Pulmonary embolism
PMN	Polymorphonuclear leukocytes
PT	Blood plasma transfusion
PVR	Pulmonary vascular resistance
PVRI	Indexed pulmonary vascular resistance
RBCT	Red blood cell transfusion
RIA	Reamer-irrigator-aspirator
S.E.M.	Standard error of the mean
SIRS	Systemic inflammatory response syndrome

SPSS	Statistical Package for Social Science
sTF	Soluble tissue factor
SvO_2	Mixed venous oxygen saturation
SVR	Systemic vascular resistance
SVRI	Indexed systemic vascular resistance
TAT	Thrombin-antithrombin complex
TCC	Terminal complement complex
TF	Tissue factor
t-PA	Tissue plasminogen activator
TR	Traditional reaming
TT	Thrombocyte transfusion
VAP	Vascular access port
vWF	von Willebrand factor

DEFINITIONS

Primary or early intramedullary nailing is defined as fracture stabilization within 24 hours after the initial trauma.

A prethrombotic state is characterized by an imbalance of hemostasis with a tendency to hypercoagulability without signs of thrombosis or evidence of fibrinogen or platelet consumption or fibrin deposition[1,2].

Systemic inflammatory response syndrome (SIRS)
The criteria for SIRS were established in 1992 as part of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference[3]. The conference concluded that the manifestations of SIRS include, but are not limited to, systemic inflammation caused by microorganisms and can thus be diagnosed in the absence of infection when two or more of the following criteria are present:

- Body temperature less than 36°C or greater than 38°C
- Heart rate greater than 90 beats per minute
- Tachypnoea with respiration rate greater than 20 breaths per minute, or partial pressure of arterial oxygen ($PaCO_2$) less than 4.3 kPa
- White blood cell count less than 4 x 10^9 cells/L or greater than 12 x 10^9 cells/L, or the presence of greater than 10% immature neutrophils

Severely injured patients
The degree of severity of injury was categorized according to the Injury Severity Score (ISS) based on the Abbreviated Injury Scale (AIS)[4,5]. The patients were defined severely injured when ISS ≥ 16[6,7].

Acute lung injury (ALI)/Adult respiratory distress syndrome (ARDS)
The current clinical standard for the diagnosis of ALI/ARDS in accordance with the 1994 American-European Consensus Criteria[8] was used. The diagnosis rests on:

- Acute onset
- Bilateral infiltrates on antero-posterior chest radiographs
- A low ratio of partial pressure of arterial oxygen to the fraction of inhaled oxygen (PaO_2/FiO_2); for ALI PaO_2/FiO_2 less than 40 kPa (300 mmHg) and for ARDS PaO_2/FiO_2 less than 27 kPa (200 mmHg)
- The absence of clinical evidence of left atrial hypertension

Sepsis

Sepsis is defined as infection plus systemic manifestations of infection[9]. The systemic response is manifested by two or more of the following conditions as a result of infection[3].

- Body temperature less than 36°C or greater than 38°C
- Heart rate greater than 90 beats per minute
- Tachypnoea with respiration rate greater than 20 breaths per minute, or $PaCO_2$ less than 4.3 kPa
- White blood cell count less than 4 x 10^9 cells/L or greater than 12 x 10^9 cells/L, or the presence of greater than 10% immature neutrophils

Pneumonia

Pneumonia is a disease marked by inflammation of the lungs and caused by viruses, bacteria, or other microorganisms. Pneumonia was diagnosed when the body temperature was greater than 38.5°C and if, in addition, one of the following criteria was met: infiltration on chest radiographs in the absence of ARDS or positive bacterial culture in bronchoalveolar lavage fluid.

LIST OF PAPERS

In the text these papers will be referred to by their Roman numerals.

I. Husebye EE, Lyberg T, Madsen JE, Eriksen M, Røise O. The influence of a one-step reamer-irrigator-aspirator technique on the intramedullary pressure in the pig femur. Injury. 2006; 37:935-940

II. Husebye EE, Lyberg T, Opdahl H, Laurvik H, Røise O. Cardiopulmonary response to reamed intramedullary nailing of the femur comparing traditional reaming with a one-step reamer-irrigator-aspirator reaming system; An experimental study in pigs. J Trauma. 2010; 69:E6-14

III. Husebye EE, Opdahl H, Røise O, Aspelin T, Lyberg T. Coagulation, fibrinolysis, and cytokine responses to intramedullary nailing of the femur. An experimental study in pigs comparing traditional reaming and reaming with a one-step reamer-irrigator-aspirator system. Injury. 2011; 42:630-637 [Epub 2010, Jul 22]

IV. Husebye EE, Lyberg T, Opdahl H, Røise O. Intravasation of bone marrow content. Can its magnitude and effects be modulated by low pressure reaming in a porcine model? Injury Suppl 2. 2010; S9-15

V. Husebye EE, Lyberg T, Opdahl H, Aspelin T, Støen RØ, Madsen JE, Røise O. Intramedullary nailing of femoral shaft fractures in polytraumatized patients. A prospective and observational study of the procedure-related impact on the cardiopulmonary- and inflammatory responses (Submitted)

SUMMARY

Background: An important part of the early trauma management is stabilization of long bone fractures. The intramedullary reaming and nailing of femoral shaft fractures has, however, been associated with pulmonary complications. These are probably due to the intravasation of bone marrow content as a result of the procedure-related increased intramedullary pressure (IMP). In order to reduce the complications, alternative systems that reduce IMP during reaming have been developed. One focus has been techniques using suction and irrigation during reaming. We wanted to evaluate the effect of using a one-step Reamer-Irrigator-Aspirator (RIA) system and compare it to a traditional reaming (TR) system, with the hypothesis that the RIA system represents a lesser operative burden than the TR system. Additionally, we wanted to evaluate to what extent cardiopulmonary function and inflammation becomes further affected by femoral intramedullary nailing (IMN) in severely injured patients, with the hypothesis that the burden of the procedure to a certain extent will drown in the massive response to the initial trauma.

Materials and Methods: In an experimental model including 28 Norwegian landrace pigs the IMP (Paper I), cardiopulmonary alterations (Paper II), numbers of lung embolisms (PE) (Paper II), inflammatory response (Paper III) and the associations between these (Paper IV) were studied during IMN and the first three post procedure days. The animals were divided into three groups, and the left intact femur was operated on either by the use of a traditional reaming (TR) system, a RIA system, or the animals were exposed to a sham operation. In 12 polytraumatized patients with femoral shaft fractures primarily intramedullary stabilized, we investigated prospectively to what extent the cardiopulmonary and inflammatory response was further activated related to reamed IMN (Paper V). The patients were followed for three days after IMN. In the postoperative course the following complications were recorded: SIRS, ALI, ARDS, pneumonia, sepsis and local wound infection.

Results: The IMP (Paper I), the numbers of PE (Paper II) and the procedure-related inflammatory response (Paper III) were reduced after RIA reaming compared to reaming with the TR device. Both reaming devices induced a hemodynamic procedure-related effect, but with no significant difference between reaming with the RIA system and reaming with a TR system (Paper II). The lowering of IMP, as obtained with the RIA system, reduced the magnitude and the effects of the bone marrow intravasation (Paper IV).

In the severely traumatized patients (Paper V) no additional procedure-related coagulation- and fibrinolytic response was found. Increased levels of TNF-α, IL-6 and IL-10, however, were found, and a transient (ns) procedure-related hemodynamic response was observed. All patients except one fulfilled the criteria of SIRS. The postoperative course was prolonged by pneumonia (7/12), ALI (3/12), ARDS (3/12) and sepsis (3/12). All the patients had additional extremity- and chest injuries.

Discussion: In healthy human individuals the amount of pulmonary vessels occluded needed to cause hemodynamic alterations has been estimated to be as high as 30 - 50%. In our experimental model the animals were young and healthy, and the embolic load induced by the procedure was probably not sufficiently massive to cause marked alterations. Additionally, the length of the pig femur is relative to body size about 50% shorter than in humans, resulting in a smaller potential volume of bone marrow content that can be extruded into the vascular system of the pigs. In our experimental model the response to reaming with the RIA and the TR systems *per se* were isolated and thoroughly investigated. In clinical practice, however, femoral shaft fractures are often caused by injuries involving high energy and are therefore associated with multiple and severe injuries. Severely injured patients already have an activated inflammatory system at hospital admission. The procedure-related hemodynamic response seen in the severely injured patients can be interpreted as a response to an embolic load, which may also contribute to the increased inflammatory response. The patients in this study had a high complications rate; the complications on which we focused were related to an increased inflammatory response.

Conclusions: Overall, in this work on reamed IMN on intact pig femora without any additional injuries or confounding artifacts, the RIA system seems to represent a reaming method with a lower operative burden than the TR system. The harmful effects of reamed IMN in severely injured patients, however, are difficult to pinpoint.

INTRODUCTION

EVOLUTION IN ORTHOPEDIC TRAUMA TREATMENT

Until the 1970s, initial treatment of fractures in polytraumatized patients was conservative, as the patients were considered physiological unstable and the operative treatment regarded as a high-risk procedure. The fear of complications related to fracture manipulation with intravasation of bone marrow content[10,11] led to delayed operative treatment and long time immobilization of the polytraumatized patients, which again resulted in high incidence of complications, mainly related to immobilization, like pneumonia, respiratory complications, pulmonary embolism, thrombembolisms and decubitus[12,13]. Several publications in the 1970s, however, changed the treatment strategy of the polytraumatized patients dramatically[14-17]. Early operative treatment of long bone fractures in severely injured patients was demonstrated to decrease mortality and morbidity, in particular the pulmonary related morbidity[12,18,19]. The theory of the severely injured patient being too weak to be treated operatively was replaced by the theory of the severely injured patient being too weak to be treated non-operatively. The principle of "early total care" was established. A modification of the principle of "early total care" was allocated in severely injured, hemodynamically instable patients, who were not expected to tolerate the extent of surgery caused by internal major fracture stabilization. In these patients the concept of "damage control orthopedics"[20] was applied; initial bleeding control, hemodynamical stabilization, and external fixation of major instable fractures, with secondary internal fracture stabilization in a stable situation. Pape et al.[21] concluded in a study that early IMN, if performed in "borderline patients", which are patients with severe trauma and associated pulmonary injury, increased the risk of pulmonary injury and the development of ARDS[21]. In several trauma centers, the strategy of "damage control orthopedics" was therefore also applied in "borderline patients". The results from a study of Morshed et al.[22] from 2009 supported a moderate delay of definitive treatment of long-bone injuries in patients with multisystem trauma also as a means of damage-control orthopedics in order to reduce adverse outcomes. Today, femoral IMN should be performed as early as possible in the well resuscitated patient.

POLYTRAUMA AND FEMORAL SHAFT FRACTURES

The appropriate timing of internal fracture stabilization has been and still seems to be controversial[23-27]. Early IMN of femoral shaft fractures (earlier than 24 hours post injury) has in some studies been associated with reduced pulmonary complications and mortality[16,18,28-30], whereas in other studies early IMN has been associated with increased inflammation, multi organ failure (MOF) and morbidity[31-34]. In patients with severe chest trauma Pape et al.[21] found a higher incidence of posttraumatic ARDS and mortality when early femoral IMN was done. In the study of Morshed et al.[22], the authors focused on the effect of treatment with internal fixation of femoral shaft fractures in patients they described to be in an "under-

resuscitated" state. They concluded that delayed internal fixation of femoral shaft fractures beyond twelve hours post hospital admission, which allow time for appropriate resuscitation in severely injured patients, reduces mortality by approximately 50%. According to the current literature severely injured patients with femoral shaft fractures should be treated with internal femoral stabilization when properly resuscitated and well perfused[22].

SUCTION-IRRIGATION-REAMING (RIA)

The main thoughts behind the development of the RIA system are:

1) reduced complications due to reduced intravasation of bone marrow content and

2) increased bone healing through medullary fat removal

Intramedullary nailing of femoral shaft fractures was introduced by Küntscher in 1939[35,36], and he was also the first to express his concerns considering events related to intramedullary procedures[37]. In the 1950's, however, the complications related to medullary content intravasation increased as Küntscher introduced motorized reaming which enabled the introduction of larger diameter nails to improve the fracture stability and reduce the hardware failure. With the hypothesis that the intramedullary fat had a negative influence on bone healing, Dankwardt-Lilliestrom et al.[38,39] performed in 1970 a randomized experimental study in rabbits comparing reaming after removing of medullary content with reaming of the bone with the medullary content left *in situ*. No medullo-hematomas were seen in the suction group where reaming was accomplished with negative pressure in front of the reamer head. The suction group had no local infections or deaths, whereas the no suction group had two deaths from infection and four deaths from massive fat embolization (11%). In these studies increased bone healing and bone vascularity was seen after reaming with suction compared to reaming without suction. The reduction of bone marrow fat was assumed to be the reason for the improved bone healing. The investigation of the suction-irrigation-reaming was continued by Stürmer[40] and was based on Pascal's Law: "Pressure applied to a confined fluid at any point is transmitted undiminished throughout the fluid in all directions and acts upon every part of the confining vessel at right angles to its inferior surfaces and equally upon equal areas". To put it simple; in order to keep positive pressure from being generated, a volume equal to or greater than the volume introduced must flow out of the chamber. Viscosity, lumen dimensions for in- and out-flow for irrigation and suction, reaming speed, diameter and design of the bore and the design and the flutes of the cutter head are important aspects involved in IMP increase. The RIA is a double lumen system allowing simultaneous flushing and suction. Fat has a higher viscosity than water and to reduce the intramedullary content viscosity, water is applied in the suction-irrigation reaming systems. To improve the out- flow, the reamer-shaft diameter was increased and a one-step reaming system was created in the RIA system.

From the time Küntscher introduced the IMN until today, the reamer head design of the reamers without suction and irrigation has changed significantly, from a piston-like reamer head design to reamer heads with increasing flutes of the cutter, allowing the cutter to better work itself through the intramedullary content and to a lesser degree push the reaming debris forward[41]. This development has also reduced the IMP during reaming significantly from levels exceeding 1000 mmHg[40,42] to levels below 350 mmHg as demonstrated in laboratory studies in Plexiglas tubes filled with a mixture of Vaseline and Paraffin oil in 1997[43]. In the same experimental setting a reamer with irrigation and vacuum demonstrated a pressure increase of 64 mmHg. In the experimental studies by Goplen et al.[44], Stürmer et al.[40] and Smith et al.[45] reaming systems that included irrigation and suction demonstrated lesser IMP increase during reaming than a TR system. However, no clinical studies, comparing IMP by suction-irrigation reaming with TR have been carried out. A recent study from Volgas et al.[46] compared the intravasation of bone marrow content, evaluated by transesophageal echocardiography, related to reaming with the RIA device with a standard reaming device. They found significantly increased amount of fat passing through the right heart during reaming with the standard reamer versus the RIA reamer.

HEMOSTASIS

Blood flow is indispensable in the normal physiologic function of the major organ systems. In order to serve its function in organ oxygenation effectively, blood must be in a fluid and non-coagulated state. The process by which the blood is maintained fluent within the vessels and at the same time prevention of excessive injury-related blood loss is called hemostasis. The balance between the forces that cause blood to coagulate and those who allow it to remain fluid is very delicate and involves several interacting systems. The hemostatic process is composed of four major events:

1. The initial phase is accompanied by vascular constriction due to contraction of the smooth muscle in the blood vessels.

2. The adhesion phase. When the endothelium is disrupted, subendothelial structures are denuded, mainly collagen, which binds circulating von Willebrand factor (vWF) and changes the conformation to allow binding of glycoprotein-1b (Gp-1b) on the surface of platelets. The Gp-Ib functions as a receptor for vWF. During this initial adhesion phase binding of platelets to denuded collagen occurs and through this binding and at the presence of additional proteins the platelets get activated. Upon activation the platelets release several proteins important for the coagulation cascade, as well as small molecular substances like adenosine diphosphate (ADP) and 5-hydroxytryptamine (5-HT), which in turn activate additional platelets. The activated platelets change their shape to accommodate the formation of a platelet plug.

3. Thrombus formation. For stabilization of the initially loose platelet plug, a fibrin mesh (called clot) forms and merges with the plug. When the plug consists of platelets it is termed white thrombus, and when red blood cells are included in the plug it is termed red thrombus.

4. Dissolution of the thrombus. For normalization of blood flow and to resume tissue repair the thrombus has to be dissolved. The dissolution of the thrombus occurs through the action of plasmin, the major fibrinolytic enzyme.

There are four major plasma enzyme systems which have an important role in hemostasis and control of inflammation. These are the clotting system (coagulation), the fibrinolytic system, the complement system and the kallikrein kinin system.

The accumulation of fibrin must be tightly regulated to prevent unnecessary blocking of blood vessels, resulting in tissues with reduced- or absent blood supply. If blood clots too easily, thrombosis may occur. When clot formation is impeded, hemorrhage may occur. Due to the need of rapid response as well as a tight regulation, the coagulation system involves a multistep cascade of enzymes with the major purpose to catalyze the next step in the cascade, ultimately resulting in cross-linked fibrin. In this way, the effect of the initial blood vessel damage can be quickly magnified, as a single enzyme at the first stage activates many copies of the enzyme at the next stage. At the same time, the many levels of interactions provide many levels of possible control processes.

For the evaluation of coagulation activation we have chosen to analyze the plasma levels of soluble tissue factor (sTF) and the thrombin-antithrombin complex (TAT). The most important coagulation activation pathway in trauma is the tissue factor pathway, which is initiated by the expression of TF. Intramedullary fat that are intravasated by bone fracturing and intramedullary procedures is a major source of TF. TAT represent a relatively stable complex consisting of thrombin and its inhibitor; antithrombin, and represents the end product of the final common coagulation pathway. Once thrombin is formed, the fibrinolytic system is triggered. We have chosen tissue plasminogen activator (t-PA) and plasminogen activator inhibitor 1 (PAI-1) as indicators for fibrinolysis and fibrinolysis inhibition, respectively. The reason why there is free t-PA in the circulation (up to 5%) is that the inhibition of t-PA by PAI-1 takes a few minutes and a small amount of t-PA is left and not yet inactivated. The coagulation- and fibrinolysis activation are further described below.

THE PLASMATIC CASCADE SYSTEMS

THE COAGULATION SYSTEM

The coagulation cascade is activated already at thirty seconds to some minutes post injury[47]. Activation of the coagulation system can follow two pathways; the TF pathway (formerly known as the extrinsic pathway) and the contact activation pathway (formerly known as the intrinsic pathway). These ultimately converge with the final common pathway that leads to clot formation. The pathways of coagulation are inhibited and activated by different enzymes.

The inhibitors of the coagulation system can be divided into three groups; the serine protease inhibitors (e.g. anti-thrombin), the vitamin K-dependent glycoproteins (protein C/S system and alpha$_2$-macroglobulin (A_2M)[48]), and the Kunitz type inhibitor group.

THE TISSUE FACTOR PATHWAY (EXTRINSIC)

The primary pathway for the initiation of blood coagulation is the TF pathway[49]. TF (also called thromboplastin, tissue thromboplastin and FIII) is not normally present in the circulation at any more than trace levels. The TF pathway of coagulation is triggered when blood gets in contact with TF that is exposed by damaged blood vessels and surrounding tissues[50,51]. TF can also be expressed intravasculary by appropriate stimulation of monocytes and endothelial cells[50,52-54]. In the presence of other plasma proteins (clotting factors); mainly Factor VII (FVII) and calcium ions, this leads to the activation of FX, which then activates the common pathway of coagulation.

Activated FVII (aFVII) circulates in a higher amount than any other activated coagulation factor, and following damage of the blood vessels, aFVII is exposed to TF, and form together the activated complex: TF-FVIIa. TF-FVIIa activates FIX and FX. The prothrombin time (PT) test and also PT ratio (= INR) are measurements of the TF pathway activation.

THE CONTACT ACTIVATION PATHWAY (INTRINSIC)

The intrinsic pathway of coagulation begins with activation of FXII (also named Hageman factor), which is released by blood platelets. FXI, prekallikrein, and high molecular weight kininogen (HMWK) are also involved in this activation pathway. The sequential activation of FXII, FXI, FIX, and FVIII in the presence of calcium ions results in the activation of FX. FVIII is essential for activation of the intrinsic pathway. The aFX then initiates the common pathway of coagulation. The contact activation pathway has a minor role in initiating clot formation, but seems, instead to be more involved in inflammation. Activated partial thromboplastin time (aPTT) is a measurement of the contact activation pathway.

THE FINAL COMMON PATHWAY

The common pathway begins with the activation of FX. The aFX, together with platelet phospholipids, aFV, Ca^{2+} and prothrombin form the prothrombinase complex, which activates prothrombin (FII) to thrombin (aFII) and prothrombin fragments 1 and 2. The primary role of thrombin is the conversion of fibrinogen into fibrin, the building block of the hemostatic plug. Additionally, thrombin activates FVIII and FV and their inhibitor protein C (in the presence of thrombomodulin) and also FXII, FXI and FIX. Thrombin also activates FXIII, which in turn participate in forming the cross-linked fibrin clot. Fibrin, as a fibrous protein with no enzyme effects, ends this cascade. The primary inhibitor of thrombin in plasma is antithrombin. Thrombin and antitrombin form the TAT complex, which is relatively stable[55,56].

Fibrin, being insoluble, sticks to platelets and endothelial cells at the site of tissue damage and forms a network that, in turn, traps other cells[47]. In addition, thrombin stimulates various cellular functions such as platelet activation, release and aggregation, smooth muscle cell mitogenesis, angiogenesis, neutrophil chemotaxis and changes in the endothelial cell shape which leads to increased outflow of albumin across the endothelial cell barrier. Following the continuing activation of the TF pathway, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX, until down-regulated by the anticoagulant pathway.

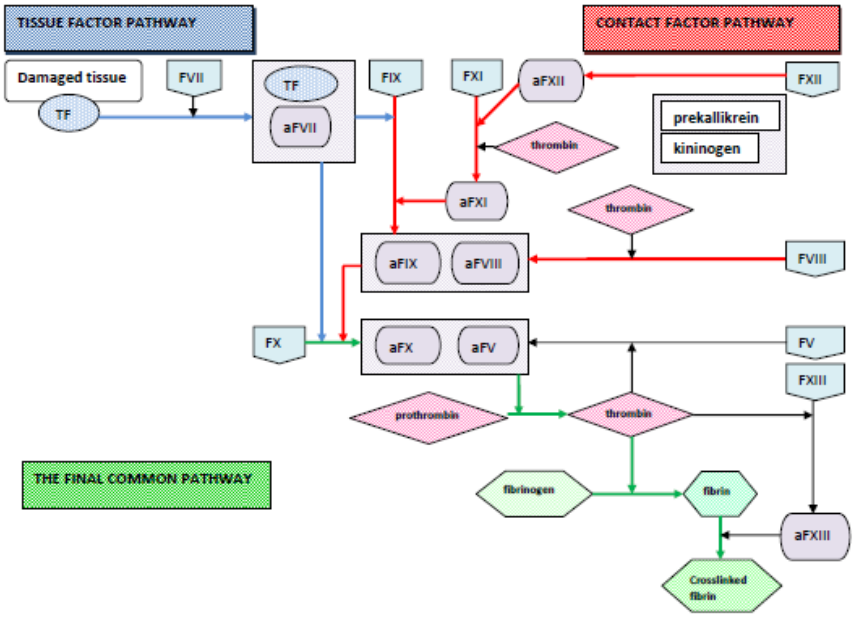

Figure 1. The figure demonstrates a schematic overview of the coagulation pathway.

THE FIBRINOLYTIC SYSTEM

Once hemostasis is established and the tissue repaired, the clot or thrombus must be removed from the injured tissue to restore normal vascular blood flow. This is achieved by activation of the fibrinolytic pathway. The process of reorganizing and decomposition of fibrin clots, the product of coagulation, is called fibrinolysis. It is stimulated by anoxia, inflammatory reactions, and other kinds of stress.

The end product of this pathway is the enzyme plasmin. Plasmin attacks fibrin at several different sites, reducing its size such that it no longer has hemostatic activity. Many fragments are formed during this process, and some retain the capacity to polymerize, thus some of the early degradation products can compete with fibrinogen for thrombin and act as inhibitors of clot formation.

Plasmin is the main enzyme responsible for the fibrinolytic process. The conversion of plasminogen into the active fibrinolytic enzyme, plasmin, occurs at the fibrin surface of the blood clot. The initiation of fibrinolysis is regulated by the concentration of active t-PA in the region of the thrombus. Plasminogen and its activator t-PA have a high affinity for fibrin and form a complex at the fibrin surface. t-PA is found in most tissues and is synthesized by endothelial cells[57,58]. The binding of the t-PA results in activation of plasmin on the cell surface to prevent, remove or split the clot formations. The concentration of active t-PA is controlled by the rate of endothelial secretion of t-PA, the rate of hepatic clearance of t-PA[59], and the inhibition of t-PA by active PAI-I[60], which is the principle inhibitor of the fibrinolysis[61]. t-PA and PAI-1 react to form an inactive t-PA/PAI-I complex. In normal individuals the half-time of t-PA is about three to four minutes and for PAI-1 it is less than ten minutes[62]. Free t-PA and also the PAI-1-complexed t-PA are rapidly removed from the circulation. The level of t-PA in the plasma is normally low, but can be increased by exercise and stress. Activated t-PA and PAI-1 can be measured in plasma.

Before effective fibrinolysis can occur, the plasmin that is initially formed must saturate the plasmin inhibitor that is bound to the fibrin. By extensive activation of fibrinolysis, plasmin may exceed the binding capacity of the main plasmin inhibitor, i.e. α_2-antiplasmin. When this happen A_2M, another inhibitor of the fibrinolysis system, may act as a second-line inhibitor.

THE COMPLEMENT SYSTEM

We analyzed the terminal complement complex (TCC) for the evaluation of the complement activation. C5a is probably the most important activation product after trauma, but is difficult to detect due to its rapid binding to leukocytes. TCC is found to be a reliable indicator of C5a [63] and has a half life of approximately 50 minutes[64]. The complement cascade consists of three pathways, and a common terminal pathway[65]:

1. The classical pathway is initiated by antigen-antibody complexes.
2. The alternative pathway is initiated by cell and tissue damage, e.g. trauma.
3. The lectin pathway is similar to the classical pathway, but is not antibody-dependent. The pathway is initiated by the binding of lectin to pathogen surfaces.

The ultimate result of either pathway is the formation of TCC. A controlled activation will destroy foreign material like microorganisms and preserve host tissues, whereas an uncontrolled systemic activation of the complement cascade may result in tissue destruction and organ failure. The alternative pathway is the most important in the trauma patients, and also for the activation seen in severe sepsis[66-68].

CELLULAR IMMUNITY

The cell-mediated immunity is an immune response that involves the activation of macrophages, natural killer cells (NK), antigen-specific cytotoxic T-lymphocytes, and their release of various cytokines in response to an antigen.

CYTOKINES

Cytokines are small protein molecules that act as signaling molecules used extensively in intercellular communication. The widespread distributions of cellular sources for cytokines include mainly endo- and epithelial cells and resident macrophages, monocytes, granulocytes and lymphocytes. The cytokine binds to a specific receptor and causes a change in function or in differentiation of the target cell. Cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. When pro- and anti-inflammatory systemic reactions are balanced, a state of homeostasis is present. The most studied cytokines are the following proinflammatory cytokines: tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) and the anti-inflammatory cytokine Interleukin-10 (IL-10).

Pro-inflammatory cytokines

Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)

Both TNF-α and IL-1β are pro-inflammatory cytokines that stimulates the secretion of other pro-inflammatory cytokines, and IL-1β is probably the initiator of the inflammatory response[69]. IL-1β is produced by macrophages, monocytes, fibroblasts and dendritic cells. The main source of TNF-α is the activated macrophages. TNF-α activates lymphocytes

(natural killer cells) that result in increased cytotoxicity. A local increase in concentration of TNF-α will cause the cardinal signs of inflammation to occur; heat, swelling, redness, and pain. TNF-α also acts on vascular endothelium and causes arterial vasodilatation in experimental animals, and it promote inflammation and thrombosis[70]. IL-1β participates in the transmigration of leukocytes to sites of infection (and the pathogens) and re-set the hypothalamus thermoregulatory center, leading to an increased body temperature. IL-1β is therefore called an endogenous pyrogen. IL-1β production in peripheral tissue has also been associated with increased sensitivity to pain associated with fever[71]. Because of the short half-life of TNF-α and IL-1β in the circulation (less than 20 minutes and 6 - 8 minutes, respectively)[72], the maximum serum concentrations decrease rapidly to not-detectable levels and could escape the fixed points of blood sampling[72]. The normal range of TNF-α is 0.3 – 5.1 pg/mL[73]. The expected level of IL-1β in human plasma is less than 1 pg/mL.

Interleukin-6 (IL-6)

IL-6 is mainly produced by T-lymphocytes and monocytes and holds both pro- and anti-inflammatory qualities[72,74,75]. The anti-inflammatory effects of IL-6 are caused by the weakening of TNF-α and IL-1β activity by increased release of IL-1 receptor antagonist and soluble TNF-receptors[75,76]. However, the main role of IL-6 is the pro-inflammatory response by inducing neutrophil activation and delaying the phagocytic disposal of aging or dysfunctional polymorphonuclear leukocytes (PMNs)[72]. IL-6 has been considered the main mediator of fever and acute inflammatory reaction after surgery[77] and also participate in inducing the hepatic synthesis of C-reactive protein (CRP)[78].

Interleukin-8 (IL-8)

IL-8, mainly produced by macrophages, is a pro-inflammatory cytokine and a potent signal molecule for activation of PMNs[72,79].

Anti-inflammatory cytokines

Interleukin-10 (IL-10)

IL-10 is an anti-inflammatory cytokine. The main source of IL-10 is probably T-lymphocytes and monocytes[72]. IL-10 is a potent inhibitor of cytokines such as TNF-α, IL-1β, IL-6, IL-8 and IL-12 and has the potential to transfer the host into an immunosuppressive state[80]. IL-10 accelerate monocyte apoptosis[72]. IL-10 inhibit the generation of free oxygen radicals and nitric oxide, which are responsible for microbacterial activity against intracellular pathogens[80,81].

INTERACTIONS BETWEEN COAGULATION, FIBRINOLYSIS, COMPLEMENT AND CYTOKINES ACTIVATION

A significant cross-talk between coagulation, fibrinolysis, complement and cytokines exists[82]. All the systems can be looked upon as partners in an inflammation response whose ultimate aim is to stabilize disturbances to homeostasis. The inflammation, when extensive, shifts the hemostatic mechanisms in favor of thrombosis. The inflammatory mediators IL-1β and TNF-α, as well as endotoxin, can stimulate the presence of accessible TF and thereby activate the TF pathway of coagulation[82,83]. Inflammatory mediators like IL-6 can increase the responsiveness of platelets to thrombin and thereby increase the thrombogenic potential[84]. TF is the most potent trigger of the coagulation system known[85].

It is well established that complement activation, directly or indirectly, promotes blood coagulation and thrombosis by increasing TF expression in various cells[86,87]. For the coagulation cascade to work effectively, phosphatidylserine is needed on the cell surface, which can be achieved by very potent platelet agonists like C5b9 (complement cascade) or the combination of thrombin and collagen[88]. Complement split products, like C3b, may stimulate the TF synthesis in monocytes which in turn induces activation of the coagulation system[89]. The complement cascade is further activated by thrombin. Thrombin is thought to primarily be involved in coagulation activation, but it seems additionally to be involved in the inflammatory response through the activation of platelets, which again release substances with pro-inflammatory qualities[90,91]. Additionally, it is well established that complement activation inhibit fibrinolysis by up-regulation of PAI-1[86,87]. Protein S may also represent a link between the coagulation and the complement systems and cytokines, and the protein C anticoagulant pathway is also participating in the modulation of inflammatory mediator responses in the endothelium[92].

THE COAGULATION-, FIBRINOLYTIC-, COMPLEMENT- AND CYTOKINE RESPONSES IN THE TRAUMA PATIENTS

Hemorrhage is the most frequent cause of preventable injury-related deaths and is commonly associated with coagulopathy[93,94]. The traditional description of trauma-associated coagulopathy views it as directly blood loss, dilution (due to fluid administration or transfusions) or dysfunction of the coagulation proteases (due to hypothermia and/or the effect of acidemia on enzyme function). The acute traumatic coagulopathy appears, however, to have an endogenous component as a result of combined hemorrhagic shock and tissue damage[95], and may develop without the exogenous factors such as hemodilution, acidemia or hypothermia[93,96-99]. In recent literature, the acute coagulopathy in trauma is described as initiated by tissue trauma and hypoperfusion and characterized by systemic anticoagulation and hyperfibrinolysis, not as a dysfunction of the coagulation proteases[100]. The term “personalized medicine” has recently been used in an editorial by Holcomb

describing the future approach to coagulopathy in trauma patients, combining the utilization of the valgus nerve, transfusion of biologically active fluids containing proteins, stem cells and reconstituted lymphilized blood products[101]. This biologically active fluid should not only increase blood pressure and reverse the coagulopathy, but also participate in tissue healing and restore vascular stability. This vision of the future is, however, based on hypothesis generating, but highly interesting, possibilities focused on interplay with endothelial barrier function (Syndecan-1), catecholamines, inflammation and coagulation[102,103]. The clinically relevant definition and the etiology of the acute traumatic coagulopathy are still not clear.

By normal hemostasis, tissue injury results in endothelial disruption with production of aFV, aFX, thrombin and aVIII. In the absence of hypoperfusion, tissue trauma activates the TF pathway, generating thrombin, which cleaves fibrinogen to form fibrin[104]. In the presence of shock and tissue damage, however, the clearance of thrombin is delayed which increases its binding to thrombomodulin (expressed by the endothelium) and the forming of complexes containing anticoagulant properties. Less thrombin is available to split fibrinogen, and thrombin-thrombomodulin complexes activate protein C, which again inhibit the TF pathway (through cofactors V and VIII) and PAI-1 (by cleaving thrombin-activated fibrinolysis inhibitor into its active form). Activation or dysfunction of platelets and endothelium and fibrinogen generation participate, together with a relative inhibition of stable clot formation by anticoagulant and fibrinolytic pathways to the coagulopathy[100].

Orthopedic surgery has in general a greater influence on coagulation activation than general surgery, due to bone traumatization which includes both local hypercoagulation in veins draining the operation fields and systemic activation of coagulation[105]. The local activation is initiated by vascular endothelial damage, exposure of subendothelial procoagulants, e.g. TF, and results in thrombin and fibrin formation and an immediate TAT response[49,95,106,107]. The systemic effects may be associated with intravasation of bone marrow and intramedullary fat, which has experimentally been demonstrated to cause systemic intravascular coagulation with aggregation of platelets and formation of fibrin clots[108]. Femoral shaft fractures and the stabilization of these have been associated with coagulopathy, but this association has been questioned.

The lungs appear to have a prominent position in the activation of coagulation in bone preparation procedures due to lodging of procoagulant material in the pulmonary capillaries. This process in turn contributes to local deposition of fibrin and formation of pulmonary microthrombi[109]. Additionally, increased TF activity of circulating monocytes, as demonstrated after elective orthopedic surgery[110], allows pericellular fibrin formation to occur, which again contributes further to a hypercoagulable state. The lodging of bone marrow-derived conglomerates in the pulmonary circulation represents the mechanical obstruction of the lung capillaries. This step is followed by a toxic and biologic effect that is

associated with vasculitis, pneumonitis, and a local inflammatory reaction[111]. Procoagulant bone marrow material occluding the pulmonary capillaries is broken down by tissue lipase to glycerol and pulmonary tissue toxic concentrations of free fatty acids[112,113]. This, in combination with other factors, results in damage of the capillary endothelium and subsequently provokes low pressure pulmonary edema and a disruption of the capillary network with confluent hemorrhage[112,113]. The polymorphonuclear leukocytes are activated by damaged endothelium. The free fatty acids increase the endothelium damage and thereby lead to a further activation of the polymorphonuclear leukocytes. ARDS is an inflammatory disease of the pulmonary parenchyma, where to bone marrow constituents may be a trigger for the develpoment[112,114,115].

Inflammation can potentiate coagulation with increased risk of thrombosis through the down-regulation of the vascular anticoagulants. Activated leukocytes and monocytes results in sequestration of platelets on which coagulation complexes can assemble, and to the triggering of coagulation and also finally to the decrease of the effectiveness of the protein C pathway as an anticoagulant[104].

In trauma patients the adversed outcome of bleeding disorders is not limited to the deaths related to acute blood loss, but include also the consequences of a prolonged shock state with coagulation activation integrated in the inflammatory response with a increased susceptibility of development of SIRS, MOF, ARDS, pneumonia and sepsis. There are, post injury, mainly two systemic mediator systems with potential to jeopardize organ function[116-118]. The first is the hyperinflammatory stage (SIRS) characterized by elevated inflammatory mediators[74,117,119,120] that, when exaggerated, results in tissue damage[121,122]. The other is the compensatory anti-inflammatory response (CARS)[117,120,123] that can transfer the injured patient into an immunosuppressive stage, with increased endotoxin tolerance and increased risk of infections[124-127]. When pro- and anti-inflammatory systemic reactions are balanced, a system of homeostasis is present. When unbalanced, the patient may be threatened by the mechanisms that should actually protect them[128]. SIRS is presumably beneficial in most patients[117], and resolves usually during the recovery.

Several events are assumed to influence the activity of the different cytokines, such as body temperature[129], soft tissue trauma[130], major fractures [31,121], severity of tissue injury[33,81,131-136], infections[137], hypovolemic shock[138], numbers of transfusions[139] and the extent of surgical procedures[131,140]. The magnitude of the inflammatory response seems to correlate with the extent of injury (operation or trauma)[33,131,132,134,136]. Secondary operations, if performed in polytraumatized patients while they still have increased level of posttraumatic inflammation, may act as second insults and precipitate late multi organ dysfunction syndrome (MODS)[34,136,141]. Cytokines are presumed to be important mediators in the development of MOF/MODS, ARDS, post injury infections and sepsis, as well as efficient predictors of outcome in severely injured patients[3,69,124,126,142-150].

Evaluating the inflammatory response to trauma and surgery still involves major challenges. The inflammatory response is thought to be a result of the summary of the local tissue injury and the immunologic reaction that is caused by local necrosis, bacteria and hypoxia. By reviewing the literature, it is still difficult to sort out the pro-inflammatory and anti-inflammatory response that is related to the initial trauma and that which is related to a surgical intervention. The effects[151-154], the origin[72,151], the mechanisms of secretion and the interactions of the various participants of the inflammatory response are partly unclear and inconsistent[73,75,80,142,155-157]. To what extent the systemic levels of cytokines represent a spill-over from a local environment is still unclear[158-160]. Several authors have demonstrated increased levels of cytokines at the site of injury or infections[158-161]. IL-6, IL-8, and IL-10 have been demonstrated to be increased in fracture/soft-tissue hematoma[159,160]. Krohn et al.[158] demonstrated increased IL-6 and stable TNF-α concentration in blood drained from the operation area when compared with the concentrations in circulating blood after major orthopedic surgery.

The time-related response of cytokines to the surgical procedure with intramedullary reaming has been studied[31-33,162], but whether the response is related to surgery or the trauma *per se* could be difficult to ascertain and most studies only measure one or a few components of a single cascade system. IL-6 is the most studied cytokine in trauma patients. A post injury association between early increased IL-6 levels, high ISS, late adversed outcome, and extent of tissue degree is well established[120,133,136,163]. IL-6 peaks 4 - 24 hours post injury and persists for 3 - 10 days[72,133]. After a surgical procedure a peak level is demonstrated 6 - 12 hours after the procedure[77]. Nast-Kolb et al.[126] presented a study on severely injured patients (mean ISS 40) with significantly more pathologic values of IL-6 and IL-8 in non-survivors post injury compared to survivors, and with a peak much broader and culminating somewhat later (18 - 32 hours) than in the patients that survived (6 - 18 hours). Giannoudis et al.[163] demonstrated increasing IL-6 levels that peaked on the first postoperative day in polytraumatized patients (NISS 31.5) in which femoral shaft fractures were initially stabilized. A strong correlation between IL-6 levels >200 pg/mL and a SIRS state and IL-6 levels >300 pg/mL and risk of complications like pneumonia, MOF and death was found. IL-6 was measured on the first, third, fifth and seventh day, no secondary IL-6 peak level was described. The study from Morley et al.[164] from 2008 provided evidence that intramedullary reaming of femoral shaft fractures can cause significantly increased local levels of IL-6. They found, however, no second-hit phenomenon with a procedure-related further elevation of peripheral IL-6 levels when measured at 24 and 72 hours post procedure. In that study all patients with femoral shaft fractures without focus on ISS level, were included. Pape et al.[141] examined blunt polytraumatized patients (ISS 34) and found high IL-6 levels on admission. Major surgery was performed on day two to four post injury, and a secondary IL-6 peak was observed at the fourth day post injury. Another study by Pape et al.[31], however, demonstrated significantly increasing IL-6 levels after operative procedures, but it is difficult to determine whether these increased levels represented an

increase related to the initial trauma or to the operative trauma. Giannoudis et al.[165] demonstrated peak IL-6 levels on the first day after admission in patients with femoral shaft fractures, but a timely relation between IL-6 levels and the operative procedure of the femoral shaft fractures was not defined, and no additional IL-6 activation related to the procedure of femoral stabilization could bee seen.

The role of TNF-α in the trauma patients has still not been definitely settled. This might be a result of the short half-life and thereby the increased possibility of missing a possible peak level related to injury or surgical procedures. Increased levels of TNF-α has been reported to be associated with morbidity and unfavorable prognosis in septic patients[161], and has been reported elevated in bronchoalveloar fluids of ARDS patients and elevated in plasma after trauma[73]. In the study by Spielmann et al.[73], no correlations were, however, found between TNF-α levels and SIRS or MODS the first six days post trauma, and further there was no correlation between systemic TNF-α levels and the total severity of the injury[73]. Serum TNF-α was unchanged in the fracture group throughout the investigation.

The demonstration of peak IL-1β levels has the same limitations as that for TNF-α; a short half-life makes it difficult to demonstrate. A brief IL-1β peak was observed at three hours after fracture in a study of Kobbe et al.[166].

Levels of IL-8 are reported elevated in bronchoalveolar lavage in patients with nosocomial pneumonia, ARDS and lung injury[143]. A large increase in circulating IL-8 is associated with the development of ARDS[133,144].

IL-10 is a potent inhibitor of production of cytokines such as IL-1β, TNF-α, IL-6, IL-8 and IL-12 and has simultaneously the potential to transfer the host into an immunosuppressed state[80,167]. Both biphasic and single peak IL-10 levels were measured 1 - 24 hours post injury and postoperatively[81,162,166], with a decrease towards baseline levels 24 - 72 hours postoperatively[80]. IL-10 response correlate with the severity of injury and posttraumatic complications[80,81,149], and is also increased in drainage fluid and serum after major orthopedic surgery[168,169]. Smith et al.[162] demonstrated that reamed femoral nailing was associated with higher IL-10 levels and with greater immunosuppression than the unreamed intramedullary nail. Despite that IL-10 is thought to have a protective effect by inhibiting the pro-inflammatory response[129], several authors have demonstrated increased mortality and morbidity related to elevated circulating levels of this cytokine[124,125,147].

Blocking or interfering with the effects of cytokines with an anti-cytokine therapy has been tried in various studies. Blocking IL-1 or TNF has been highly successful in patients with rheumatoid arthritis, inflammatory bowel disease, or graft-vs.-host disease. Such strategies have, however, been without success in humans with sepsis or trauma[119]. Blocking of the complement cascade in animal models of ischemia-reperfusion injuries have shown promising results[170,171], but human studies are lacking. Recent studies has described the

mechanisms were valgus nerve stimulators inhibit cytokines[172], but the application of this knowledge is not investigated related to trauma.

The complement activation occur very early after trauma (within 30 minutes post injury)[173], mainly via the alternative pathway[174] , and has been implicated in the pathogenesis of several inflammatory and immunological diseases, including SIRS, ARDS, sepsis and MOF[175]. In a study on polytraumatized patients TCC correlated with ISS, and C3a were significantly increased in non-survivors when compared to survivors[176]. No studies have demonstrated a relation between IMN and complement activation, but in relation to elective orthopedic surgery increased TCC levels are found systemically and in drained blood at the location of the operative procedure[158].

ORTHOPEDIC SURGERY AND CARDIOPULMONARY COMPLICATIONS

Severe injuries including long bone fractures result in extensive tissue injury, intravasation of bone marrow content and coagulation and inflammation activation. A cardiopulmonary response is also present[177,178]. The exact nature, as well as the sequelae of this response might be unclear, but they are likely to participate in the later development of life-threatening complications.

Pulmonary fat embolization was found in 62 - 96% of the cases in autopsy reports from trauma patients with fractures[179-181]. In an experimental model a biphasic embolic load has been demonstrated after fracturing of the bone and the subsequent reaming and nailing of the fractures. The first and the highest embolic shower was related to fracturing, and the secondary peak embolic load was observed related to the reaming procedure[182]. In the studies of Wenda et al.[183,184] an IMP greater than 50 mm Hg correlated with embolic showers. In experimental models, an IMP higher than 1000 mm Hg in the femoral cavity has been demonstrated[40,42]. Intramedullary femoral reaming and nailing may induce intravasation of bone marrow contents as a result of increased IMP[183-186]. These embolic elements may lodge in the pulmonary circulation and result in cardiopulmonary complications[114,186-189].

Intravasation of bone marrow content is experimentally proven by histological investigations and sonographic visualization of embolic echoes after manipulation of bone marrow and increased IMP[183,184]. The magnitude of hemodynamic and pulmonary changes after PE has been demonstrated to be a function of both size and numbers of the emboli in addition to the underlying pre-existing state of cardiopulmonary function[187,190]. The amount of emboli needed to cause a detectable cardiopulmonary dysfunction has still not been established. The vessels in a normal pulmonary circulation are easily dilated when the pressure increases, therefore both mean pulmonary artery pressure (MPAP) and pulmonary vascular resistance (PVR) may remain almost unchanged despite widespread vascular occlusion. In older

literature it has been estimated that as much as 30 - 50% of a previous normal pulmonary circulation in humans has to be occluded before hemodynamic alterations can be detected [191]. The hemodynamic consequences of microscopic pulmonary embolism and microthrombosis associated with conditions like ALI and ARDS could be circulatory failure due to the effect of increased MPAP on the right ventricle[186]. The right ventricle can be expected to fail when 75% of the pulmonary vasculature becomes obstructed[192]. Such failure leads to end-diastolic dilatation and displacement of the septum wall, which results in reduced end-diastolic filling of the left ventricle and further aggravation of circulatory failure[190,193]. Pulmonary dysfunction following intravasation of bone marrow contents is also reported to be aggravated by secondary problems such as hypoxia, hypovolemia, pulmonary vasoconstriction, pre-existing pulmonary disease and massive blood transfusions. Bone marrow fat is primarily filtered by the lungs, which are the first organ affected when no intracardial shunt is present. If PE causes massive occlusion of the lung vessels intraoperatively, it can result in immediate heart failure. Both the mechanical and the biochemical pulmonary effects of PE result in an immediate and serious impairment of oxygen transfer from the airways to the hemoglobin. The hypoxemia results from an increased pulmonary venoarterial admixture (pulmonary shunt). Perfusion disturbances through arteriolar and alveolar capillary occlusion by emboli can account for the increased alveolar dead space (ventilated and nonperfused alveoli), but not for hypoxemia. The mixing of arterial and venous blood in the lung may have other causes like extensive alveolar consolidation by hemorrhage or edema producing non-ventilated but perfused alveoli.

In a recently published review of 20 experimental studies on IMN as a second hit phenomenon, the authors concluded that *i)* a second hit consisting of reamed IMN resulted in a consumption of coagulation factors, *ii)* hemodynamic function parameters were transiently affected, and *iii)* pulmonary function parameters were only affected when the initial injury included a pulmonary injury[194].

AIMS OF THE STUDIES

The aims of the present studies were*:*

1. to compare the procedure-related effects of intramedullary reaming with the RIA system with a TR system in an experimental model and
2. to explore the additional procedure-related effect of reamed IMN in severely traumatized patients

In Paper I we evaluated the IMP increase during reaming with the RIA and the TR technique.

In Paper II we evaluated the effect of reaming with the RIA and TR technique on cardiopulmonary function parameters and numbers of embolisms.

In Paper III we evaluated the effect of reaming with the RIA and TR technique on the coagulation-, fibrinolytic- and cytokine responses.

In paper IV we investigated the associations between low-pressure intramedullary reaming, pulmonary embolism, cardiopulmonary alterations and inflammatory response.

In paper V we evaluated the procedure-related effects of reamed IMN in severely injured patients.

METHODOLOGICAL STRATEGIES

MATERIALS

EXPERIMENTAL STUDIES (PAPER I-IV)

We used Norwegian landrace pigs, age 3-4 months, weight 30-40 kilogram (15 female, 13 male animals). The animals were from the same farm. The genetic aspect was not investigated.

Twenty-eight animals were used in the experimental studies. The same study animals were used in Paper I-IV. In ten animals we used the RIA system, in another ten the TR system was used, and eight animals constituted the control group. Two of the animals in the RIA group were used in a pilot study, and had a shorter stabilizing period after soft tissue preparation before intramedullary surgery was performed. One animal in the TR and one in the control group were not included in the analyzes due to anesthesia-related events. One animal in the RIA and one in the TR group died of massive bleeding, due to dislocation of catheters after the intramedullary surgery was performed. The group distribution of the animals is not a true randomized inclusion, even though a randomly allocation to the groups was described in Paper I, Paper II and Paper III. The first two animals were used as pilots for testing the RIA system in our model. Thereafter, the procedure changed for every third animal (TR or RIA) and the last 8 animals were the controls. The animals were, however, picked randomly at the farm and delivered at Institute for Experimental Medical Research (IEMR). A flow-chart of included and excluded animals are given in Figure 2.

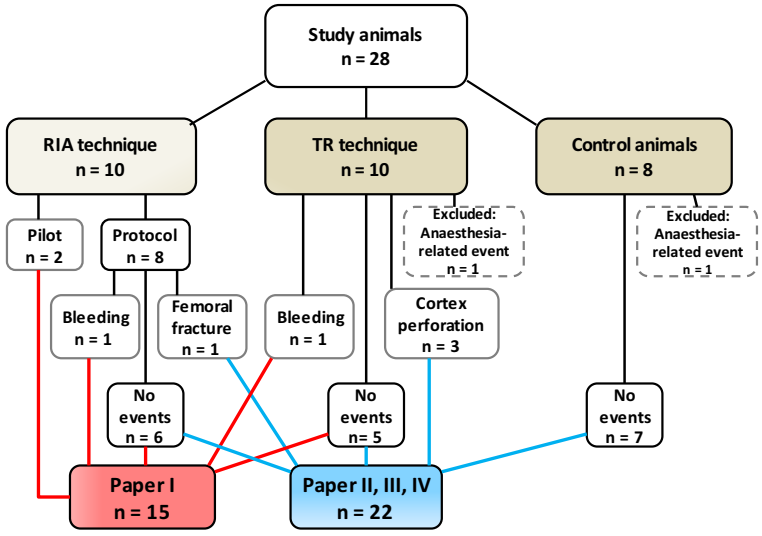

Figure 2. Flow-chart of included and excluded animals in paper I-IV.

Paper I: The two pilot animals were included in this paper. One animal in the TR group was excluded due to anesthesia-related events prior to intramedullary surgery. The two animals with larger bleeding were included (one in the TR and one in the RIA group). One animal in the RIA group was excluded due to a perioperatively inflicted femoral fracture, as were three animals in the TR group due to perioperative perforation of the distal medial femoral cortex. After the exclusion of these animals the RIA group consisted of nine animals and the TR group of six animals. No control animals were included.

Paper II and Paper III: Six of the 28 animals were excluded due to anesthesia-related events prior to operation, exsanguinations or a shorter than protocol stabilization time, leaving 22 animals for final analyzes; 8 in the TR, 7 in the RIA and 7 in the control group.

Paper IV: Six of the 28 animals were excluded due to anesthesia-related events prior to operation, exsanguinations or because they were used in the pilot study, leaving 22 animals (8 in the TR, 7 in the RIA and 7 in the control group) for final analyzes of correlations. When analyzing correlations related to IMP measurements the control group was not included.

The physiology of the digestive system in pigs is rather similar to that of humans. The adult porcine heart is approximately the size of the human heart and is also anatomically and physiologically similar to the heart of the humans[195].

The porcine hemodynamics is similar to that of human beings. The lung tissue volume of small and large animals makes up nearly the same fraction of body volume of 6% [196,197]. Mammals in general, have a tidal volume of one-tenth of their maximal inspiration lung volume, irrespective of their body size[196]. Human tidal volume is approximately 500 ml[198] and at maximal inspiration the gas volume is approximately 5000 ml.

The porcine model, with 75 ml blood per kilo body weight, offers sufficient quantity of blood for sampling. Knowledge of the species-specific normal blood parameters is essential when selecting the appropriate model for studies. Porcine models have been widely used in research on coagulation and fibrinolysis since the components of the porcine and the human coagulation- and fibrinolytic systems are assumed to be similar[195], but porcine plasma is hypercoagulable compared with human plasma[199-201]. The hypercoagulability is probably not solely caused by increased coagulation activity, but also by a relatively modest fibrinolytic activity or even absent increase in fibrinolysis. TAT level reflects the formation of thrombin (prethrombotic state) and is an indicator of the inhibition of thrombin by its natural inhibitor antithrombin III, and can be detected in several species (e.g. mice, dogs, sheep, pigs[2]) with the analyzing kit based on polyclonal antibodies against human antithrombin III and prothrombin used in the present studies. Roussi et al.[199] concluded that TAT could be used to follow the modifications of blood coagulation activation during experimental procedures in pigs. The porcine levels of TAT and fibrinogen, are elevated compared to human levels[201], but have a wider range of variation[199,200]. At sampling, care should be taken not to

manipulate catheters and samples and thereby cause elevated levels. And related to interventions, it is of importance to carefully follow the time schedule. In the study of Velik-Salchner et al.[201] from 2006, normal mean TAT levels in porcine blood was 20.2 µg/L while the mean TAT level in human blood was 2.6 µg/L. Roussi et al., on the other hand, found normal porcine plasma to have TAT levels of 9.3 µg/L[199]. Measurements of PAI-1 plasma concentration in pigs has demonstrated a wide range of levels[199]. t-PA plasma concentration in pigs is similar to the human concentration of t-PA[199]. The application of immunoreagents, developed for applications in humans, can therefore be used for detection of both of t-PA and PAI-1 activity[199]. Commercial kits for the TF (thromboplastin) levels in pigs are not available, and lack of cross-reactivity between pigs and humans makes it not possible to use human assays[202].

CLINICAL STUDY (PAPER V)

During the inclusion period from May 2003 to December 2004, 43 patients between 18 and 65 years of age, with femoral shaft fracture, were admitted to Oslo University Hospital, Ullevaal. By selecting patients that were severely injured and suitable for early IMN, 15 patients were eligible for inclusion, 12 patients were included. A detailed description of the patient material is given in Figure 3.

Oslo University Hospital, Ullevaal, was in 2003/2004 the local trauma hospital for 250 000, the major trauma hospital for 550 000, and the trauma referral hospital for 2.5 million people. Annually, approximately 1100 patients were enrolled in the hospital-based trauma registry (numbers from 2008). Approximately 40% of these patients were severely injured. Empirically, 80% of the patients with femoral shaft fractures admitted to Oslo University Hospital, Ullevaal, were severely injured patients.

A recently published study from Sweden[203], describing as many as 6409 patients with femoral shaft fractures, demonstrated an incidence of all femoral shaft fractures in all age groups to be 10 fractures in 100 000 persons per year. The two major mechanisms of injury were falls on the same level (50%) and transport accidents (17%). A significant number of fractures occurred among elderly patients after low-energy trauma. The material on femoral shaft fractures in the present study differed significantly from that study.

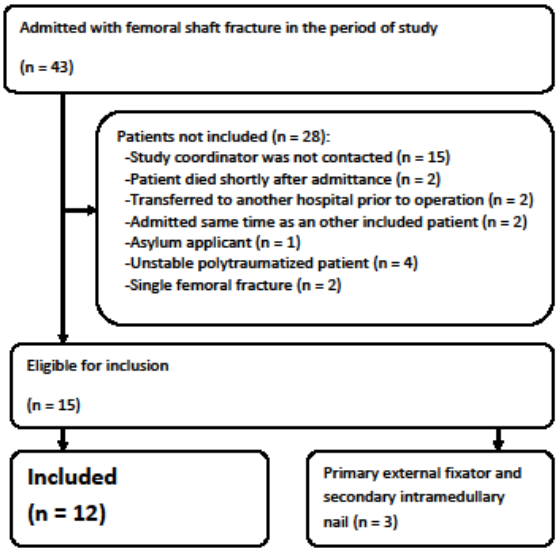

Figure 3. The flow chart describes which of the patients with femoral shaft fractures admitted to the hospital in the given period of time were included in the study.

STUDY DESIGN

INTERVENTION

EXPERIMENTAL STUDIES (PAPER I-IV)

Before starting of the study we evaluated the technical applicability of the available RIA equipment in the chosen research animal. RIA reamers were available with the smallest diameter of 12 mm, which reduced the choice of experimental models to large animals. We used young pigs with a body weight of 30 to 40 kilograms. These animals, however, had an open distal femoral epiphysis. To determine the localization of the epiphysis and the shape and dimensions of the femur, radiographs of the femur of one pig used for other research purposes were taken (Fig. 4a and 4b). Furthermore, the femora of two other pigs were excised and divided longitudinally for easier identification of the epiphysis that was located at the level of the proximal lateral edge of the patellofemoral cartilage (Fig. 4c) at a distance of 11 - 12 centimeters from the top of the trochanter. This was important to ensure the placement of the intramedullary pressure catheter above the epiphysis in order to secure correct IMP measurements and to ensure no risk of damaging the catheter during reaming. As no x-rays or fluoroscopy were used during surgery, the placement of the catheter above the epiphysis had to be correlated to anatomical landmarks. The lateral edge of the patella was palpated and a longitudinal incision made. In the mid-line of the distal femur at the level of the most prominent part of the proximal, lateral edge of the patellofemoral cartilage a hole was drilled at the desired location.

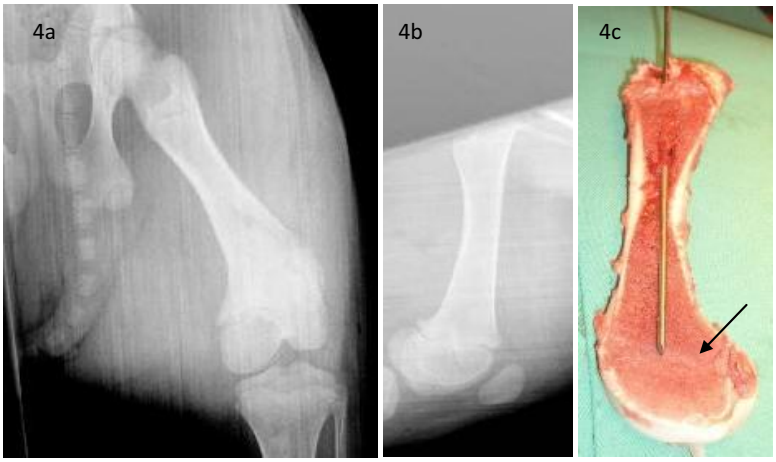

Figure 4. Radiographs (4a and 4b) of a pig femur and a longitudinally divided pig femur (4c) with distal femoral epiphysis (black arrow).

Prior to the present studies, we devided the femur longitudinally after reaming with the RIA device to evaluate the size of the reamer related to the size of the femur (Fig. 5). The planning of the study included also a surgical approach.

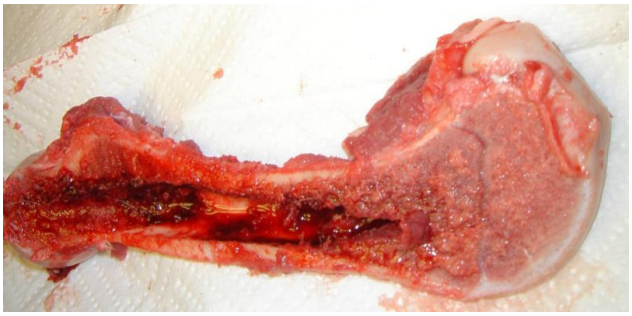

Figure 5. Longitudinally divided pig femur post RIA reaming.

Anesthesia

We chose a model in which the animals were extubated and kept alive for three days after surgery. In acute experiments on large animals, an open tracheotomy is normally performed. As the animals were to be extubated oral intubation was chosen. During the two hours post procedure period, the surgical anesthesia was maintained by infusions of propofol (Diprivan®, 20 mg/ml, AstraZeneca, Caponago, Italy) and fentanyl (Fentanyl®, 50 µg/ml, Alpharma, Linz, Austria). This method, which is similar to that utilized in many trauma patients, gives the opportunity to regulate the level of analgesia and consciousness within short time. The pigs need, however, larger doses of drugs than humans to achieve adequate effect. The possible effect of propofol or fentanyl on blood coagulation, fibrinolysis or cytokines is not known. In the current study all animals received the same anesthesia. As propofol is suspended in 10% soy bean oil, the most concentrated solution was used to avoid interference of exogenous fat with the parameters to be measured.

Insertion of a femoral vein catheter

An 18-gauge catheter was inserted through a cut-down in the left femoral vein. This catheter was used for perioperative venous blood sampling.

Vascular Access Port, VAP

Two hours after the insertion of the nail, an artery catheter that was inserted in the carotid artery was cannulated under the skin and connected to a VAP that was placed in a subcutaneous pocket in the posterior area of the neck. This procedure made it possible to sample blood from awake animals without sedation. The catheter was installed arterially to minimize the risk of blood clotting and occlusion of the catheter. The VAP was flushed with 5 ml of a solution consisting of 500 ml sodium chloride (0.9%), 500 ml Macrodex® (60 mg/ml with sodium chloride) (Pharmalink AB, Upplands Väsby, Sweden) and 15 ml heparin (Heparin Leo®, 5000 U/ml, LEO Pharma A/S, Ballerup, Denmark).

Pulmonary artery (PA) catheter

Installing a catheter in the pulmonary artery is the only possible method of continuous and direct monitoring of the parameters needed for the calculation of pulmonary vascular resistance (PVR). In the first two animals, the pilot, we introduced the flow-directed PA catheter (744HF75, Swan-Ganz CCOmbo CCO/SvO_2 catheter 7.5F, Edwards Critical-Care Division, Irvine, CA, USA), by using a percutaneous technique via the right femoral vein (the left femur was operated on). This procedure proved cumbersome; we therefore changed the site of introduction to the left internal jugular vein using an open technique with a midline incision at the anterior aspect of the neck.

Intramedullary pressure monitoring

The insertion of the pressure monitoring catheter was performed following the information obtained during the pilot phase. A transducer tipped pressure monitoring catheter, zero adjusted, was inserted and fixed with a compression cap. The tip of the catheter was placed about 2 mm beyond the end of the bolt. The catheter was connected to both a display unit for continuous graphic observation of the IMP and to a monitor (Camino1MPM1, San Diego, USA) for recording of pressure levels at fixed time points. The measurement range of the monitor was -10 to +250 mm Hg; the average pressure of the last 0.5 seconds is given instead of the continuous pressure. We recorded the IMP as described in Table 1. The graphic pressure observations were used to evaluate the recorded reaming time with a pressure greater than 50 mm Hg. The time between the reaming steps was not included in the reaming time in the TR group. In particular during the first reaming steps in the TR group very high peak IMP levels for very short time periods (not measurable) were observed. Whether these represent artifacts or are caused by embolic masses, is unclear.

Reaming and nailing of the femur

We used no radiography during the reaming and nailing procedure. The location of the femur and the anatomical characteristics of the pig were studied prior to the start of the study. Reaming and nailing of left femur was performed with the pig in the right lateral position. The trochanteric fossa was prepared through a longitudinal incision; the medial cranial part of the femoral biceps muscle was partly divided, carefully avoiding the sciatic nerve. The marrow canal was then opened with an awl, a guide wire introduced intramedullary and antegrade reaming was performed. The femoral neck is very short in the pig, and the opening of the femoral canal has to be located as close to the cartilage of the femoral head as possible. In the RIA group, a 12 mm reamer head (Fig. 6a and 6b), which is the smallest available, was used. The application of smaller animals or thinner femora appears as a difficult if not impossible exercise if the intension is to keep the outer cortex intact. The reamer was connected to a suction tube (Fig. 6c) with an internal lumen of 5.5 mm and a suction force of 30 mm Hg. In the TR group, the nailing was performed after sequential reaming with increasing steps of 0.5 mm in reamer diameter (9.5 - 12 mm). A standard Groβe-Kempf nail, cut at a length of 10 cm, with a diameter of 10 mm was introduced.

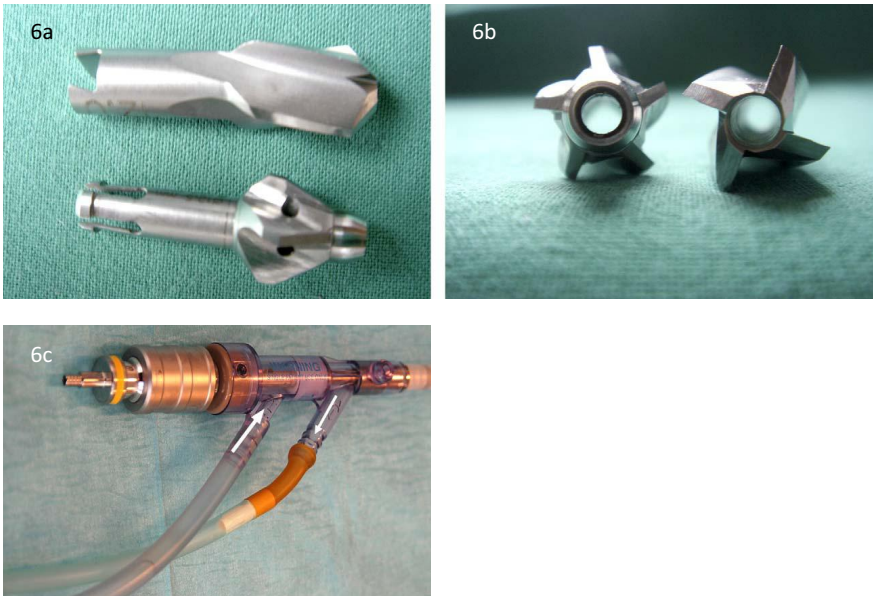

Figure 6. The figure shows the RIA reamer head (Fig. 6a and 6b, below and left) and the traditional reamer head (Fig. 6a and 6b, above and right). The RIA is demonstrated on Fig. 6c with a connection to a flushing tube (white, thick arrow) and a suction tube (white, thin arrow).

Lung specimens

The animals were sacrificed and autopsied on the third postoperative day. The lungs were excised immediately after the death. Two tissue samples were harvested from the periphery of each lung lobe; one anteriorly and one posteriorly located, and two histological sections made from each sample which resulted in twenty sections per animal. The hearts were examined to exclude the presence of a patent foramen ovale or any right-to-left intracardiac shunt. The lung specimens were fixed in 4% paraformaldehyde. For light microscopy the specimens were washed and processed using a tissue processor, starting with 50% ethanol and finally being embedded in paraffin wax. Histological sections were cut at a thickness of 4 μm and were conventionally stained with hematoxylin and eosin and covered with mounting medium (Permount, Fischer Scientific Ltd., Ottawa, Canada). The size of the emboli (in large, medium or small pulmonary arteries, arterioles, venules or veins) and emboli content (fat, fibrin, bone marrow, bone fragments and combinations of these) were noted. The total area of the lung histology preparations was calculated using Auto Sketch 6.0 (1998) planimetry program. The incidence of embolisms was expressed as numbers of emboli per square centimeter lung area.

CLINICAL STUDY (PAPER V)

In the clinical study the inclusion criteria were severely injured patients (ISS ≥ 16), aged 18 - 65, with a femoral shaft fracture suitable for early IMN and no previous femoral shaft fractures or operative interventions in the femur. The inclusion in the study did not influence the choice of treatment method or the time to perform the operative procedure.

Pulmonary artery catheter

In 8/12 patients the PA catheter was introduced via the subclavian or jugular veins. In one patient an attempt of catheter insertion was unsuccessful. In three patients time constraints precluded catheter insertion previous to the surgical procedures.

BLOOD SAMPLING AND HANDLING

In order to discriminate between a local activation at the location of intramedullary intervention and the additional pulmonary activation, we sampled arterial-, mixed venous- and femoral vein blood simultaneously. Mixed venous blood from the pulmonary artery contains venous blood from both the upper and lower body, in contrast to samples withdrawn from a central venous line, where the samples reflects the venous drainage from the upper body.

EXPERIMENTAL STUDIES (PAPER I-IV)

For the evaluation of coagulation TAT was studied. Fibrinopeptide A and Prothrombin fragments 1 and 2 are not available as commercial porcine kits, neither is sTF.

For the evaluation of fibrinolysis and inhibition of fibrinolysis t-PA activity and PAI-1 activity were studied. The soft tissue damage during surgery is minor, but an effect on at least the coagulation and fibrinolytic system is to be expected. In the pilot study we used a stabilizing period of only 45 minutes and received elevated baseline TAT levels. We decided therefore a stabilizing period of 75 minutes after the soft tissue preparation.

We analyzed the terminal complement complex (TCC) for the evaluation of the complement activation.

For the evaluation of pro- and anti-inflammatory cytokines, we chose to analyze and evaluate changes in circulating IL-1β, TNF-α, IL-6, IL-8 and IL-10. The sandwich ELISA technique can specifically detect and quantitate the levels of soluble cytokines.

Time	Surgical procedure	Blood samples	Blood sample location			Cardiopulmonal recordings	IMP registration
			a	m	v		
A	Baseline	Coagulation, fibrinolysis, cytokines, blood gas	x	x	x	CO, SVR, PVR, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference	x
	At opening of the femur with an awl						x
	Intramedullary guide-wire						x
B(A+ 15 min)	After reaming	Coagulation, fibrinolysis, cytokines, blood gas	x	x	x	CO, SVR, PVR, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference	x
C(B+ 7 min)	After nail insertion	Coagulation, fibrinolysis, blood gas	x	x	x	CO, SVR, PVR, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference	x
	10 minutes after nail insertion						x
D(C+ 30 min)	30 minutes after nail insertion	Coagulation, fibrinolysis, cytokines, blood gas	x	x	x	CO, SVR, PVR, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference	
E(D+ 90 min)	2 hours after nail insertion	Coagulation, fibrinolysis, cytokines, blood gas	x	x	x	CO, SVR, PVR, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference	
F(E+ 4 hours)	6 hours after nail insertion	Coagulation, fibrinolysis, cytokines	x				
G(F+ 18 hours)	24 hours after nail insertion	Coagulation, fibrinolysis, cytokines	x				
H(G+ 24 hours)	48 hours after nail insertion	Coagulation, fibrinolysis, cytokines	x				
I(H+ 24 hours)	72 hours after nail insertion	Coagulation, fibrinolysis, cytokines	x				

Table 1. The table shows time points for blood sampling and the different sampling locations, cardiopulmonary recordings and intramedullary pressure registration in the porcine model; 75 minutes after completion of soft tissue preparation (baseline) (A), after reaming (B), immediately after insertion of the nail (C), and at 30 minutes (D), two (E), six (F), 24 (G), 48 (H) and 72 (I) hours after insertion of the nail.

CLINICAL STUDY (PAPER V)

The first arterial blood samples were withdrawn in the emergency room at hospital admittance.

For the evaluation of coagulation pathway, sTF and TAT were studied. For the evaluation of fibrinolysis and inhibition of fibrinolysis t-PA and PAI-1 were studied. We used specific assays for the evaluation of human t-PA activity and antigen and PAI-1 activity, which were all cross-reactive with porcine components.

The terminal complement complex (TCC) was analyzed for the evaluation of the complement activation.

Pro- (IL-1β, TNF-α, IL-6, IL-8) and anti-inflammatory (IL-10) cytokines were analyzed with colometric sandwich ELISA (IL-8) or chemiluminescent ELISAs that can specifically detect and quantitate concentrations of soluble cytokines. The samples were first analyzed using the normal colorimetric sandwich ELISA, but since several samples had to be diluted because they were out of the range of the assay, we choose to switch to the chemiluminescent ELISAs with increased sensibility and a wider dynamic range to eliminate the need for extra dilutions. We reanalyzed the already analyzed samples. The results used in the study, however, were the results from samples analyzed with the same kit.

CARDIOPULMONARY FUNCTION RECORDINGS AND CALCULATIONS

As pulmonary embolism was a central issue in these studies, the alterations in the pulmonary circulation were important. To obtain the parameters needed for the calculation of PVR (according to the formula: [MPAP – pulmonary capillary wedge pressure (PCWP)] / cardiac output (CO) x 80), we inserted a flow-directed PA catheter in the pulmonary artery. Accurate measurement of CO is important for the PVR calculations, thermodilution by a PA catheter is still considered the gold standard for clinical CO measurements. Figure 7 demonstrates the association of the different parameters that can be monitored and used for evaluation of cardiopulmonary function. The PA catheter was installed through the right ventricle and into the pulmonary artery where the MPAP was measured. When the balloon at the end of the catheter is inflated, the pressure sensor at the end of the catheter will measure the pressure in the pulmonary vein which corresponds to PCWP.

The PA catheter was connected to a Vigilance monitor for continuous peroperative monitoring of CO, mixed venous oxygen saturation (SvO_2) and body core temperature. Mean artery pressure (MAP), MPAP, central vein pressure (CVP) and heart rate were monitored continuously, PCWP was measured intermittently, and SVR and PVR were calculated from the measured parameters. Alveolar PaO_2 was calculated by the equation $P_AO_2 = ((P_B-P_{H2O}) \times F_iO_2 – P_aCO_2/0.8)$ (P_B = barometric pressure, P_{H2O} = Water vapor pressure, 47 mmHg, 0.8 is

the normal gas exchange ratio or respiratory quotient), and the $P_AO_2 - P_aO_2$ differences were calculated.

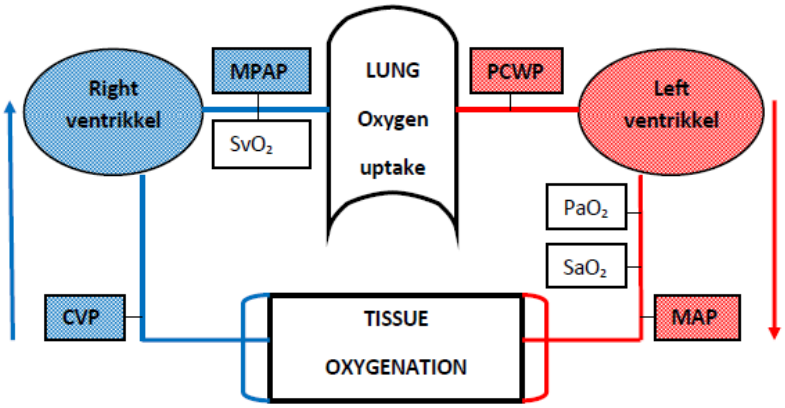

Figure 7. The figure demonstrates the location and associations of pressures and gas saturations in the circulation.

STATISTICAL METHODS

All statistical analyzes in this thesis were performed with the Statistical Package for Social Science (SPSS Inc, Chicago, IL, USA) version 12.0 (Paper I), version 15.0 (Paper II) and version 16.0 (Paper III, Paper IV and Paper V).

EXPERIMENTAL STUDIES (PAPER I-IV)

All animal studies should be designed to include a minimal numbers of animals considering both the ethical and the economical aspects. Reliable sample size calculations are often difficult to achieve because neither the magnitude of the effect size nor variability of the data is known when planning the study. The sample size calculations depend on the effect size, the variability, significance level and desired power. When not using large animals, this can be estimated using a pilot study. A statistical sample size calculation, using sample size formulas, was not performed. We used the advice from the University Hospital Senior Medical Statistician who recommended at least 6 - 8 “successful“ study animals in each study group. To allow for a 20% drop-out rate, 10 animals were initially included in each reaming group. In most animal studies, as in the present studies, the information on numbers needed is lacking. Only in one experimental study[204] referred to in Paper I-V statistical power was described. The authors set the statistical power of 50%, given the small size of samples. When making calculations posterity e.g. for the presence of PE (Paper II),

previous research could give us an indication of the amount of pulmonary emboli, the way of describing the amount, however, was not equivalent. But, if we in Paper II chose a clinically relevant difference of 0.100 (which we in fact do not know), included eight animals, used $p \leq 0.05$, the power (when using "nomogram for calculating sample size or power" by Altman[205]) was 80 - 85%. Most of the previously conducted experimental studies[42,45,183,184,204,206-212] have relatively few individuals in each study group (≤ 6 individuals), several studies have a poorly or missing description of statistics[183,184,207,209-211,213] and several studies had a short observation period (< 60 minutes)[45,211,214]. We used the hypothesis that any major differences could be demonstrated when including a minimum of 6 – 8 animals in each study group. The numbers of animals needed should be kept low, but sufficient. In this study we might have a type 2 error, and thereby possible false nonsignificant results, or results that were not clear.

Paper I

In this study we compared the IMP increase between the two reaming groups. We had no control group. Kolmogorov-Smirnov test of normality demonstrated normally distributed values. The results were presented as means and standard error of the mean (S.E.M.). The differences between the groups were demonstrated by using independent samples t-test. Differences were considered significant at P values ≤ 0.05.

Paper II

In this study we investigated the numbers of PE and hemodynamic and pulmonary alterations in two different reaming groups and a control group. The data were presented as group means and S.E.M. Independent samples t-test was used when comparing numbers of emboli, body weights, carcass weights and lung weights between the groups. Repeated measurement (mixed-between-within) analysis of variance (ANOVA) was used as a multivariate test when comparing the groups simultaneously at several time points. The values used for this test were the differences from baseline level. Differences were considered significant at P-levels ≤ 0.05. Statistical analysis of differences between the animals that died of PE and the rest of the TR group were not performed due to small number of animals.

Paper III

In Paper III the coagulation-, fibrinolysis- and cytokine activation after reaming with two reaming methods and a control group were studied. Independent samples t-test were used when comparing groups at various time points. Repeated measurement (mixed-between-within) ANOVA was used as a multivariate test when comparing the groups simultaneously at several time points. Differences were considered significant at P-levels ≤ 0.05. When values were below detectable levels, the lower detectable levels were chosen for the

statistical analysis. Statistical analysis of differences between the animals that died of PE and the rest of the TR group were not performed due to small number of animals.

Paper IV

In this paper we investigated the relationship and the strength of relationship between the results collected in Paper I, Paper II and Paper III. The correlations were obtained by the use of Pearson product-moment correlation coefficient (r). The statistical significance of r was provided. For IMP and number of PE absolute values were used, and for cardiopulmonary alterations and inflammatory-, fibrinolytic- and coagulation responses the difference (Δ values) from baseline levels (A) were used. Correlations were considered significant at P-levels ≤ 0.05 and considered strong when r = < -0.500 or > 0.500.

Paper V

In this study we further explored the effect of IMN on different parameters measured over time. Normally distributed data were presented as group means and S.E.M. Paired-samples t-test was used for evaluation of increasing or decreasing levels between two measuring points. Non-parametric statistics were used when the scores, evaluated by Kolmogorov-Smirnov test and histogram, were not normally distributed, as given for red blood cell transfusions (RBCT), coagulation-, fibrinolysis- and cytokine activation. We used median levels for presentation. Wilcoxon Signed Rank Test was used as the non-parametric test describing increasing and decreasing levels between two measuring points. Differences were considered significant at P-levels ≤ 0.05.

ETHICAL CONSIDERATIONS

Animal models allow investigations that would otherwise be impossible in humans. The use of animals in research must be conducted according to the recommendations in order to avoid pain, suffering, distress, discomfort and harm to the animals. When performing an animal study both ethical and economical aspects have to be considered carefully. The numbers of animals needed should be kept low, but sufficient. In paper I-IV we have used an experimental model and these studies were conducted in accordance with “Regulations on Animal Experimentation”[215] under The Norwegian Animal Welfare Act and approved by the Norwegian Animal Research Authority.

The clinical study was approved by the Regional Ethics Committee and the Norwegian Data Inspectorate. Informed written consent was obtained from all subjects included in Paper V. In this study we did discuss the need for the insertion of a PA catheter. The seriousness of the injuries made some kind of hemodynamic monitoring necessary anyhow, and as the effect of PE was an essential issue, we decided to insert the PA catheter. The direct complication rate related to PA catheters is low, estimated to 0.1 – 0.5%[216]. The most common serious complications related to PA catheter include arrhythmias, injury to the

lung, thromboembolism, and sepsis. PA catheterization performed by experienced team is in multicentre studies demonstrated to be a safe procedure[216,217]. When this study was planned, the use of PA catheters was more widespread than it is today. Today, methods analyzing the pulse-contour are frequently used for the estimation of CO. As this method alone does not measure the lung venous pressure, it does not give us the information necessary to calculate the pulmonary vascular resistance.

SUMMARY OF THE PAPERS

PAPER I

The influence of a one-step reamer-irrigator-aspirator technique on the intramedullary pressure in the pig femur

A one-step RIA was developed to reduce the IMP during the reaming procedure of long bones. This study compared the IMP during reaming with the RIA system with the IMP during reaming with a TR system in pig femora. Fifteen Norwegian landrace pigs were included in the final analysis. With a transducer-tipped pressure monitoring catheter the IMP in intact pig femora, reamed with either the one-step RIA device (n =9) or a TR system (n = 6), was monitored before the start of the reaming procedure, during the reaming and nailing procedure and 10 minutes after the nail was inserted. The highest IMP during reaming was 33 ± 8 mm Hg (mean ± S.E.M.) in the RIA group and 188 ± 38 mm Hg (mean ± S.E.M.) in the TR group. In conclusion the use of a one-step RIA technique in the pig femur induced significantly lower IMP increase than the use of a TR system.

PAPER II

Cardiopulmonary response to reamed intramedullary nailing of the femur comparing traditional reaming with a one-step reamer-irrigator-aspirator reaming system; an experimental study in pigs

Intramedullary reaming and nailing increase IMP and cause intravasation of bone marrow content. Intravasated bone marrow content may result in pulmonary microembolism and cardiopulmonary alterations. The clinical manifestations of intravasated bone marrow content depend on the emboli size and amount. In this study we evaluated the cardiopulmonary alterations related to the two different reaming devices; the RIA system and the TR system. Hemodynamic and pulmonary effects were investigated during the reaming and nailing procedure and for two hours postoperatively. The animals were sacrificed after 72 hours and lung tissue collected for evaluations of amount of pulmonary emboli (PE). For the final analyzes seven animals were included in the RIA group, eight in the TR group and seven in the control group. We found no significant differences in hemodynamic or pulmonary function parameters between the reaming groups. The number of emboli per cm^2 lung tissue was approximately 50% higher in the TR group than in the RIA group. This difference, however, was not sufficient to cause statistically significant group differences for cardiopulmonary function parameters. We concluded that intramedullary reaming and nailing induced a hemodynamic procedure-related effect, but with no significant difference between reaming with the RIA system and reaming with a TR system.

The numbers of PE were higher (ns) in the TR group, and in the TR group two animals died of PE. No animals died of PE after reaming with the RIA system.

PAPER III

Coagulation-, fibrinolysis-, and cytokine responses to intramedullary nailing of the femur. An experimental study in pigs comparing traditional reaming and reaming with a one-step reamer-irrigator-aspirator system

Operative procedures on trauma patients represent a second insult and the extent of the surgical procedures influences the magnitude of the inflammatory response. We investigated whether the RIA system would cause a lesser inflammatory response than the TR system. Coagulation-, fibrinolysis- and cytokine responses were studied in arterial-, mixed venous- and femoral vein blood peroperatively and until two hours after the nail was inserted for demonstration of local, pulmonary and systemic activation of the cascade systems. At 6, 24, 48 and 72 hours after the nail was inserted arterial blood samples were withdrawn. In both reaming groups significantly procedure-related activation of coagulation and fibrinolysis were demonstrated. The local and pulmonary activation of coagulation and fibrinolysis were more pronounced related to TR than related to reaming with the RIA system. The cytokine response, mainly represented by increased levels of IL-6, showed significantly higher peak levels in femoral vein blood in the TR group. The arterial IL-6 levels were higher (ns) than the mixed venous levels indicating an additional pulmonary activation of IL-6. We concluded that a procedure-related inflammatory response was demonstrated in both reaming groups and that this inflammatory response was more pronounced in the TR group than in the RIA group.

PAPER IV

Intramedullary fat embolization. Can its magnitude and effects be modulated by low pressure reaming in a porcine model?

Increased IMP may result in intravasation of bone marrow content and the extent of clinical manifestations related to the intravasation probably depends on the quantity of bone marrow content entering the blood system. In Paper I-III we demonstrated increased IMP, increased inflammatory response and increased numbers of emboli (ns) related to TR compared to reaming with the RIA system. In this study, however, we investigated the correlation between IMP increase, regardless of type of reamer, and inflammatory response, pulmonary embolization and cardiopulmonary alterations. A strong correlation was found between increased IMP and increased coagulation- and cytokine responses. Increased IMP

did not correlate to numbers of emboli. Number of emboli correlated strongly with increased coagulation- and cytokine responses. A correlation between coagulation and cytokine activation was also demonstrated. No clinical relevant correlations were observed between increased IMP or numbers of emboli and changes in cardiopulmonary function parameters. In this study we confirmed the connection between increased IMP, increased coagulation activation and cytokine activation and the magnitude of PE. We concluded that by lowering the IMP (as obtained with the RIA system) the magnitude and the effects of the bone marrow intravasation were reduced.

PAPER V

Intramedullary nailing of femoral shaft fractures. Does the procedure have an impact on the cardiopulmonary- and inflammatory responses in severely injured patients?

Early IMN of femoral shaft fractures in severely injured patients is an important procedure in early trauma management, but has also been associated with increased inflammation, MOF and morbidity. In this study we evaluated to what extent the cardiopulmonary function parameters and inflammation becomes further affected by primary femoral IMN in severely injured patients. Twelve severely injured patients with femoral shaft fractures that were primarily stabilized with a reamed intramedullary nail were included in this study. The patients were mean 27.6 years old. ISS was 31. Chest abbreviated injury scale (AIS) was 3.7. All the patients had additional extremity- and chest injuries. In 8/12 patients a PA catheter was inserted. Cardiopulmonary function parameters were recorded or calculated, and serial blood samples were collected for evaluation of coagulation-, fibrinolysis- and cytokine activation peroperatively and the first three postoperative days. The clinical course and complications were recorded. No increased coagulation- or fibrinolysis activation related to the intramedullary procedure could be demonstrated. Peak levels of TNF-α, IL-6 and IL-10 were delayed compared to other studies. We did not find evidence for an additional pulmonary component in the increased cytokine levels as we suggested in the experimental study in Paper III. A transient procedure-related effect on PVRI was present. The additional IMN-induced traumatic changes in severely injured patients seemed to be modest compared to the total extent of injury. The transient procedure-related hemodynamic response was probably a result of the procedure-related bone marrow content intravasation. The coagulation- and fibrinolysis response to the IMN seemed to drown in the extensive general trauma response. The late cytokine peak levels might be related to the IMN procedure and the postoperative course was prolonged by pneumonia (7/12), ALI (3/12), ARDS (3/12) and sepsis (3/12). All patients except one fulfilled the SIRS criteria. These severely injured patients had a high incidence of inflammatory related complications.

GENERAL DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Blood sampling and analyzing assays (Paper III, Paper IV and Paper V)

One problem concerning several previous studies from other research groups is the missing information of detectable levels of the cytokines. In the recent years several improvements of the commercial assay kits, such as high-sensitivity kits, have become available. Studying the literature on this issue, one of the challenges is to know what we are comparing. Former studies that showed no differences from normal values should be interpreted with care. Most authors mention the sensitivity of the assay kits; fewer authors mention the range or the lower detectable level of the assay kits. Available porcine assay kits are not as sensitive as modern, high sensitivity human kits, and therefore some of the changes that could have been detectable in human, are below the detectable concentration in the porcine kits.

For analyzes we primarily chose the assays that our laboratory normally uses, but as several levels were above/below the range of these assays, we reanalyzed the plasma samples with more sensitive assays with a wider range. Surprisingly, when correlating the first values with the reanalyzed values, greater discrepancies than expected were found also when the values were within the range of both assays. This information was communicated to the producing company. We used the same assays for all animals, and we used the same assays for all included patients.

Another unclarified aspect is the possible effects of freezing and thawing on the stability of plasma cytokines. In the present studies errors caused by repeated thawing cycles seem unlikely because the samples were thawed and frozen again only once. Another aspect that might have influenced the cytokine levels in the clinical study is that the samples were frozen for a long time before they were analyzed. This could have reduced the levels[218].

In the porcine study the animals were conscious when samples were obtained the first, second, and third day. They received no tranquillizing drugs prior to the blood sampling. The effect of stress on the cytokine levels is unknown.

The possible circadian variation in cytokine levels is insignificant in the porcine model (Paper III)[219] as the animals were operated at the same time of the day. In the clinical study (Paper V), this, however, could have an influence.

Krohn et al. reported no effect on the systemic concentration of cytokines after autologous transfusion of shed washed erythrocytes during an operation[158]. In the clinical study (Paper V) the amount of blood products are listed, but the material is not adjusted for the different blood products administration.

The pig femur (Paper I-IV)

Since the length of the pig femur relative to body size is about 50% shorter than in humans (approximately 12-13% of the body length versus 25% in humans) the potential volume of bone marrow content that can be extruded into the vascular system of pigs is significantly smaller in the pigs than in humans. Additionally, the trabecular bone is denser, the cortical bone less dense, and the venous circulation probably better in the porcine femur when compared to human adult bone[220]. Both a wider and shorter bone and denser trabecular bone are conditions that probably lower the IMP increase during intramedullary reaming and thereby also the intravasation of bone marrow content. These reflections make the possibility to observe an embolic load comparable to reaming procedures in humans unlikely. However, no other animal model, with the exception of maybe a monkey model (which today is seldom used in experimental models), could better reflect the human conditions.

Another topic of discussion is whether to use a fracture- or osteotomy model or a model with an intact femur. The length of the pig femora in the young animals used for the present studies was approximately 15 cm, and the distance from fossa piriformis to the distal femoral epiphysis was 11-12 cm[41]. An osteotomy or a shaft fracture may act as a vent and allow bone marrow contents to leak into surrounding tissues. The maximal pressure effects occur as the reamer or nail (by unreamed nailing) enters the distal main fragment[40]. The short femur and additionally the location of the femur in the pig make a fracture model complicated and unreliable. On the other hand, a fracture may represent a “first-hit” in the pulmonary circulation, which might have made a procedure-related increase in PVR easier to detect. An osteotomy of the femur as a fracture substitute, however, is possible, but with the length of the porcine femur, the created IMP would probably be too low to test the study hypothesis. As the effect of bone marrow embolization during reaming and nailing was one goal of our study, we do not think that inducing a femoral fracture prior to surgery would have increased the pathophysiological changes as it would probably lower the amount of bone marrow content extruded into the vascular system. In an experimental study of femoral IMN on intact and fractured canine femora the release of triglycerides was greater in a non-fracture model[221], suggesting more bone marrow content intravasation in intact femora. To achieve a maximal bone marrow content intravasation the bone in the large animal model must be kept intact and the reaming and nailing of the femur must include the distal part of the femur[222]. These considerations made us decide to use a non-fracture model.

Pulmonary injury (Paper II)

In a recently published review of 20 studies on intramedullary nailing as a second hit phenomenon in experimental studies, the authors summarized the results that second hit represented by reamed IMN resulted in a consumption of coagulation factors. The hemodynamic function parameters were transiently affected, and the pulmonary function parameters were only affected when the initial injury included a pulmonary injury[194]. In several studies a pulmonary contusion was included in the experimental design[222-224], but with huge variations in time from pulmonary contusion to IMN. A pulmonary contusion added in the experimental model could have affected the pulmonary circulation, which might have made a procedure-related increase in PVR easier to detect. As we employed a chronic model, inducing a chest injury would have made postoperative extubation difficult and would probably cause pain and suffering for the animals. The other choice could have been to include a pulmonary injury and reduce the study period, but in that case the studies of coagulation-, fibrinolytic- and cytokine activation had to be restricted to some hours after the surgical procedure.

Age of the animals (Paper I)

We used young animals with a body weight of 30 to 40 kilograms. These animals, however, had open epiphysis. The difference in bone density between cortical and trabecular bone in these animals is lesser than in adult human bone. This could contribute to the cases of bone perforation during the reaming and nailing procedure. The use of older animals of the same race is no alternative, due to the size of the animals. We could have used minipigs, but they are significantly more expensive. Another alternative could be the use of sheep, if the sheep femur has the dimension to allow intramedullary reaming with a 12 mm reamer head without perforating the cortex.

Pulmonary embolism (Paper II)

One of our goals was to create a model in which the amount of bone marrow content intravasation made the pathophysiological consequences of such intravasation possible to detect and evaluate.

This evaluation, however, is complex; both as to the estimation of volume of bone marrow material entering the circulation, and the evaluation of the potential harmful effects. The actual presence and estimation of the volume of bone marrow content in the blood stream could, in a semiquantitative manner, be detected by echocardiographical verification or examination of the fat emboli material withdrawn from venous blood during IMN. Due to the complexity of this experimental model, such investigations were not included.

Retrospectively, obtaining an estimate of the volume of embolic material would have been interesting. We tried to evaluate the amount of bone marrow content intravasation by examining the numbers of emboli per square centimeter lung tissue. Exactly this method has not previously been described. In the literature several methods of analyzing the amount of pulmonary emboli have been applied, and the methods are often poorly described. In experimental models the highest concentration of emboli was found within the first postoperative day, with emboli equally distributed to all lung lobes[207,225]. With regard to the highest concentration of emboli alone, the preferable time for lung tissue collection, would be a day after IMN. By doing that, however, we would probably miss the peak level of e.g. IL-6.

Pulmonary artery catheter (Paper V)

Pulmonary artery catheterization was introduced into clinical practice in 1970[226]. The use of PA catheters to guide clinical strategy has been a matter of discussion. At the PA catheter consensus conference in 1997, a recommendation was to use the PA catheters in trauma, the use of PA catheters was uncertain when related to SIRS, shock and hemodynamic instability[227]. The PA catheter has the ability to accurately assess CO, MPAP and PCWP, which are the needed values for PVR calculation ([MPAP- PCWP]/CO x 80). Even though MPAP as well as CO can be estimated by echocardiography, the PA catheter is the only method offering exact measurement of all parameters involved in PVR calculation. In cases where PVR is no central issue, monitoring or calculation of CO is usually sufficient for the evaluation of oxygen supply and systemic vascular resistance (SVR) ([MAP - CVP]/CO x 80). Today, frequently applied methods used for the estimation of CO are based on analyzing the pulse-contour of the arterial pressure (PICCO, LIDCO, Vigielo FlowTrack), but still, when questions related to PE and increased PVR is a vital issue, the gold standard is the monitoring and calculations achieved by the PA catheter. The analyzes of the pulsecontour is sufficient for CO estimation in most patients, but are not useful in patients with heart rhythm disorders, and this method is not useful for the calculation of PVR. As the effect of pulmonary embolism was an essential issue, and thereby the PVR, we decided the use of a PA catheter.

DISCUSSION OF MAIN RESULTS

The main results in this thesis are:

From the experimental studies:

- TR resulted in higher IMP than RIA reaming (Paper I)
- TR resulted in 50% higher numbers of pulmonary emboli than RIA reaming (Paper II)
- Reaming and nailing with both reaming systems resulted in cardiopulmonary changes with no significant differences between the reaming systems (Paper II)
- Two animals in the TR group died of PE, none in the RIA or the control group (Paper II and III)
- The procedure-related coagulation- and fibrinolysis activation was more pronounced in the TR than in the RIA group (Paper III)
- IL-6 peaked 6 hours post procedure and the local activation of IL-6 was more pronounced in the TR group compared to the RIA group (Paper III)
- Numbers of emboli and IMP correlate with coagulation- and fibrinolysis activation (Paper IV)
- Numbers of emboli, IMP and cardiopulmonal function parameters were not correlated (Paper IV)
- Coagulation- and fibrinolysis activation correlated with cytokine activation (Paper IV)

From the clinical study:

- The total systemic trauma response was mainly related to the initial trauma (Paper V)
- No discernible coagulation- and fibrinolysis response was demonstrated related to IMN in severely injured patients (Paper V)
- In the severely injured patients a transient procedure-related hemodynamic response was demonstrated (Paper V)

Increased IMP associated with femoral reaming and nailing has been seen as a cause for the systemic complications. Küntscher introduced IMN as early as 1939[35,36]. He was also the first to address the systemic problems related to this procedure. Since that time, evolutions within several fields related to trauma patients and equipment have taken place. The

intramedullary procedure-related complications are probably, due to better reaming devices, reduced compared to earlier. Previous studies have described IMP greater than 1000 mm Hg[40,42]. In Paper I the IMP, however, was mean 188 mm Hg. The intensive care medicine has achieved enormous improvements[228,229]. In the 1970s more aggressive attitude towards operations on severely injured patients which until then had been treated conservatively pending better times[17,230], was launched. The reamer head design has changed from a piston-like design to reamer-heads with deep grooves and also taper formed designs, unreamed nails had been introduced, and during the last year's different devices including suction and irrigation during reaming have also been introduced. All the equipment modifications have been made in order to reduce the temperature and IMP increase during reaming of long bones. Stürmer was the first to describe the use of suction and irrigation already in 1986[40,231]. In 2003 the next papers on suction or suction and irrigation were published[232,233]. A poster presentation of a cadaver study demonstrating low pressure reaming by RIA was presented in 2002[234]. The present paper (Paper I), however, is the first study published which has demonstrated lower pressure by reaming with the RIA device than with a TR device. Several publications concerning the RIA equipment and the application of it has recently been published[43,44,46,235-246]. The correlation between IMP and embolic showers has been proven, and embolic showers have been demonstrated in the circulation already at an IMP at 50 mm Hg[184,187].

Increased IMP, which is thought to be responsible for the systemic response related to IMN, was studied in Paper I, the results of a reduction of IMP were studied in Paper II and III. Intravasation of bone marrow content and the microembolization of bone marrow content are observed after fractures [179-184] and intramedullary bone surgery[182-184]. No other studies on the RIA device concerning numbers of PE, coagulation-, fibrinolysis- and cytokine activation or cardiopulmonary alterations has been published prior to the start of this study. Other reaming systems including suction and irrigation published before Paper I have not included these aspects. Pape et al.[247] showed in 2005 that the systemic IMN-related effects could be minimized by the use of RIA in sheep which were also exposed to unilateral pulmonary contusion. In our experimental model, both reaming systems were associated with pulmonary emboli, cardiopulmonary alterations, coagulation-, fibrinolysis- and cytokine activation, with mainly more pronounced changes in the TR than in the RIA group.

The extent of danger of performing IMN in severely injured patients has frequently been a topic of discussion, both prior to[19,28,187,211,214] and following the present studies[22,25,30,194,248]. In Paper V, an IMN-related response mostly seemed to drown in the huge response from the initial trauma. The remaining question, however, is, was the IMN procedure responsible for the increasing cytokine levels and thereby participating to the very high complication rate? The TNF-α, IL-6 and IL-10 responses did not correlate to observed peak levels in other publications[72,77,133,162,166] and they appear to rise too early to be caused by the coming complications.

MAIN CONCLUSION

The main conclusions of this thesis are:

1. Reaming with the TR involved greater systemic response than reaming with the RIA reamer.

2. The additional effects of IMN in severely injured patients are difficult to sort out.

In Paper I, we found that the IMP increase during reaming with the TR technique was significantly higher than during reaming with RIA technique.

In Paper II we found that both reaming systems induced a hemodynamic procedure-related effect with no significant difference between the systems. The numbers of PE were higher (ns) in the TR group, and in the TR group two animals died of PE. No animals died of PE in the RIA group.

In Paper III we found a procedure-related inflammatory response in both reaming groups, and this response was more pronounced in the TR group.

In paper IV we found that by lowering IMP, the magnitude and the effects of the bone marrow intravasation was reduced.

In paper V we found a transient procedure-related hemodynamic effect of the IMN in severely injured patients and a delayed, when compared to other studies, peak cytokine levels. We could not demonstrate a procedure-related response on coagulation- and fibrinolytic systems.

FURTHER RESEARCH

Future investigations have to show whether the use of less invasive surgical techniques, such as RIA, will decrease the rate of postoperative organ failure, in particular lung complications, in the trauma patient.

Whether a selective manipulation of immunologic systems may reduce the systemic inflammatory response and improve the outcome of severely traumatized patients warrants further studies

The most frequently used guides for resuscitation are markers for global circulation like blood pressure, urine output, heart rate, base deficit and serum lactate levels[22]. These can, however, not rule out occult tissue hypoperfusion. In the future better markers of coagulation and inflammation[141,249] and/or more sensitive measurements of tissue oxygenation[250] may guide the optimal time for orthopedic procedures more accurately.

In the human study, Paper V, a notable high frequency of inflammatory-related complications, such as pneumonia, SIRS, ARDS, ALI and sepsis, were observed. The early postinjury role of bacteria in these patients needs to be studied with more sensitive and better analyzing methods. If an association to bacteremia is found, targeted treatment at an early stage could reduce morbidity and severe complications in these patients.

The role of endogenous and/or exogenous stem cells in repairing tissue damage is unclair, might be of importance and needs to be defined. Beiermeister et al.[251] demonstrated the mobilization of hematopoietic progenitor cells into peripheral blood and than sequestered in damaged tissue post trauma. In our animal model we included blood sampling for the evaluation of stem cell related to the reaming methods, as the mobilization of stem cells to the circulation possibly may have an impact on bone healing as well. However, the applied method evaluating side population cells was not successful.

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ERRATA

Page 10: **"**The acute onset of bilateral infiltrates on antero-posterior chest radiographs" is changed to:

- Acute onset
- Bilateral infiltrates on antero-posterior chest radiographs

Page 20: The primary inhibitor of thrombin in plasma is antithrombin. Thrombin and antitrombin form the TAT complex, which is relatively stable[49,55,56]. Ref. 49 is removed.

Page 33 and 34: Figure 2 that was presented to the opponents and the text on page 33 and 34, described that:"One animal in the RIA and one in the control group were not included in the analyzes due to anesthesia-related events. Two animals in the TR group died of massive bleeding". This is not correct. The correct description of the groups is: One animal in the TR and one in the control group were not included in the analyzes due to anesthesia-related events. One animal in the RIA and one in the TR group died of massive bleeding.

Page 39: The size of the reamers was described as (9 mm - 12 mm) the correct size is (9.5 - 12 mm).

Page 50: The time points for arterial blood sampling are changed from 24, 48 and 72 hours to 6, 24, 48 and 72 hours.

Page 57: Küntscher introduced IMN in 1939 (not 1950) and the references are from 1948 and 1950. Ref. 36 is added.

PAPER I-V

I

II

III

IV

Title:

Intramedullary nailing of femoral shaft fractures in polytraumatized patients. A prospective and observational study of the procedure-related impact on cardiopulmonary- and inflammatory responses.

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Abstract:

Background: Early intramedullary nailing (IMN) of long bone fractures in severely injured patients has been evaluated as beneficial, but has also been associated with increased inflammation, multi organ failure (MOF) and morbidity. This study was initiated to evaluate the impact of primary femoral IMN on coagulation-, fibrinolysis-, inflammatory- and cardiopulmonary responses in polytraumatized patients.

Methods: Twelve adult polytraumatized patients with femoral shaft fractures were included. Serial blood samples were collected to evaluate coagulation-, fibrinolytic-, and cytokine activation in arterial blood. A flow-directed pulmonary artery (PA) catheter was inserted prior to IMN. Cardiopulmonary function parameters were recorded peri- and postoperatively. The clinical course of the patients and complications were monitored and recorded daily.

Results: Mean ISS was 31 ± 2.6. No procedure-related effect of the primary IMN on coagulation- and fibrinolysis activation was evident. TNF-α increased significantly from 6 hours post procedure to peak levels on the third postoperative day. IL-6 increased from the first to the third postoperative day. IL-10 peaked on the first postoperative day. A procedure-related transient hemodynamic response was observed on PVRI two hours post procedure. 11/12 patients developed SIRS, 7/12 pneumonia, 3/12 ALI, 3/12 ARDS, 3/12 sepsis, 0/12 wound infection.

Conclusion: In the polytraumatized patients with femoral shaft fractures operated with primary IMN we observed a substantial response related to the initial trauma. We could not demonstrate any major additional IMN-related impact on the inflammatory responses or on the cardiopulmonary function parameters. A transient cardiopulmonary response related to IMN was demonstrated. Delayed arterial TNF-α, IL-6 and IL10 peak levels were observed, and it could be questioned whether these were related to the procedure.

Trial registration: ClinicalTrials.gov Identifier: NCT01042132.

Keywords: intramedullary reaming, intramedullary nailing, inflammatory response to trauma, femoral shaft fracture, polytrauma, coagulation and fibrinolysis in trauma patients

Background

Early intramedullary nailing (IMN) has, in several studies, been associated with reduced pulmonary complications and mortality[1-6], whereas in other studies early IMN has been associated with increased inflammation, multi organ failure (MOF) and morbidity[7-9]. Severe trauma results in a generalized inflammatory response that can pose a threat to the organism. In trauma patients, operative procedures, such as IMN, represent a second insult, and the ideal time for such treatment, to pose as little harm as possible, has been a topic of discussion[1, 10]. The impact of primary femoral IMN on coagulation-, fibrinolysis-, inflammatory- and cardiopulmonary responses in polytraumatized patients was analyzed in the present study, with the intention to study the additional procedure-related impact.

Methods

Material

This study was approved by the Regional Ethics Committee (ref. 02066). Informed consent or assent was obtained from the patients or their representative prior to inclusion in this study. In cases when the patients were unable to give personal consent at hospital admission, the personal consent was given posterity.

Patients between 18 and 65 years of age, with femoral diaphyseal fracture suitable for initial IMN within 24 hours post injury were included. The exclusion criteria were previous femoral shaft fracture, pathological fracture, femoral deformities or pregnancy.

Between May 17th 2003 and December 6th 2004 43 adult patients between 18 and 65 years of age with femoral shaft fractures were admitted to the hospital. An overview of the patients and the inclusion process is given in Figure 1. We compared the results from the severely

injured patients subjected to primary IMN with three patients that were initially externally fixated within 24 hours post injury and had secondary IMN after 6 - 10 days.

Details concerning age, gender, mechanisms of injury and associated injuries were recorded. The injury severity was assessed according to the Abbreviated Injury Scale (AIS) and scored by a certified AIS-registrar[11, 12]. The overall severity of the injuries was calculated according to the Injury Severity Score (ISS) and New Injury Severity Score (NISS)[13, 14]. The time from injury to hospital admittance and to IMN, AO classification, Gustilo Anderson classification of open fractures, and amount of blood products transfused were recorded. The clinical course of the patients and complicationswere monitored and recorded daily. Number of days in the intensive care unit (ICU) was also noted. Severely injured patients were defined as patients with ISS $\geq$ 16[15, 16]. The definitions of systemic inflammatory response syndrome (SIRS) and sepsis were according to the Consensus Conference of the American College of Chest Physicians and the Society of Critical Care Medicine of 1992[17]. The definitions of acute lung injury (ALI) and ARDS were according to the American-European Consensus Conference on Acute Respiratory Distress Syndrome of 1994[18].

Cardiopulmonary monitoring

An 18-gauge arterial line was placed in the radial artery in all patients for continuous recording of arterial pressures. A flow-directed pulmonary artery (PA) catheter (744HF75, Swan-Ganz CCOmbo CCO/SvO_2 catheter 7.5F, Edwards Critical-Care Division, Irvine, CA, USA) was inserted prior to IMN in 8/12 patients for continuous peri- and postoperative monitoring of indexed cardiac output (CI) and mixed venous oxygen saturation (SvO_2). Mean pulmonary artery pressures (MPAP), central venous pressures (CVP) and pulmonary capillary wedge pressures (PCWP) were also monitored. In the remaining four patients a pulmonary

catheter was not inserted due to unsuccessful procedure (n = 1) or logistical difficulties (n = 3). Indexed systemic (SVRI) and pulmonary vascular resistance (PVRI) and alveolo-arterial oxygen ($P_AO_2 – P_aO_2$) differences were calculated using kPa as the unit for gas pressure, the latter by the equation: $[(95 \times FiO_2) – (PaCO_2/ 0.8)] – PaO_2$. Hemodynamic and lung function parameters were recorded at time points as described in Table 1. The arterial and PA catheters were removed when the patient left the ICU, or at latest on the third postoperative day. In the patients initially treated with external fixation and nailed secondarily, the PA catheter was inserted prior to secondary nailing. Registrations and calculations were performed during IMN and the three following days or for a shorter time period when the patient left the ICU earlier.

Surgical procedure

All patients had general anesthesia with the exception of one, who received spinal anesthesia. A standard antegrade technique was used for reamed IMN (Bicut Intramedullary Reamer System, Stryker, Trauma GmbH, Schöhnkirchen, Germany), and the femur was sequentially reamed 1-2 mm greater than the applied nail diameter. In 11/12 patients a T2 nail (Stryker) was used. In one patient a Groβe-Kempf nail (Stryker) was used. In three patients primary external fixation (Hoffman II, Stryker, Kalamazoo, Michigan) with secondary IMN (T2 nail) was performed. Cephalotine 2 g x 3-4 (Keflin®, 50 mg/ml, Lilly, Florence, Italy) was given intravenously as antibiotic prophylaxis according to the hospital routines.

Blood sampling

Hemoglobin (Hb) was measured in peripheral blood. Arterial- and mixed venous blood gas analyzes were collected according to Table 1. Serial blood samples were collected for determination of coagulation-, fibrinolytic-, complement- and cytokine activation in arterial

(sample time A-I) and mixed venous (sample time B-I) blood (Table 1). At sampling the first 3 - 4 ml blood was always discarded. The samples were collected in Stabylite® (Biopool AB, Umeå, Sweden) tubes, and vacutainer tubes containing ethylenediaminetetraacetic acid (K2EDTA) or 1/10 vol 0.13M trisodium citrate. The tubes were gently turned, placed on ice and centrifuged within 30 minutes at 2000 g for 12 minutes at 4°C. Plasma was aliquoted, transferred to 1.5 mL polypropylene tubes, and stored at -70° C until assayed. The samples were thawed only once. In the three patients who were initially externally fixated and subsequently nailed, blood samples were withdrawn related to both procedures.

Assays

Citrated plasma was used for the determination of thrombin-antithrombin-complexes (TAT) (Enzygnost TAT Micro, Behringwerke AG, Marburg, Germany, cat# OWMG15), plasminogen activator inhibitor (PAI-1) (TriniLIZE PAI-1 activity, Trinity Biotech, Jamestown, NY, USA, cat# T6004), tissue plasminogen activator (t-PA) antigen (TriniLIZE t-PA antigen, Trinity Biotech, cat# T6001), soluble tissue factor (sTF) (Imubind TF Elisa, American Diagnostica Inc., Greenwich, CT, USA, cat# A845) and terminal SC5b-9 complement complex (TCC) (by using an enzyme-linked immunosorbent assay (ELISA) as described by Mollnes et al.[19]). Stabylite plasma was used for determination of t-PA activity (TriniLIZE t-PA activity, Trinity Biotech, cat# T6002).

The following cytokines were analyzed in EDTA plasma by using commercially available ELISA kits; TNF-α (R&D Systems Europe, Abingdon, UK, Human TNF-α QuantiGlo Chemiluminescent Sandwich ELISA, sensitivity < 0.45 pg/mL, cat# QTA00), IL-6 (R&D, Human IL-6 QuantiGlo Chemiluminescent Sandwich ELISA, sensitivity 0.156 pg/mL, cat# Q6000B), IL-1β (R&D, Human IL-1β QuantiGlo Chemiluminescent Sandwich ELISA, sensitivity < 0.4 pg/mL, cat# QLB00), IL-8 (R&D, Human CXCL8/IL-8 Quantikine

colorimetric Sandwich ELISA, sensitivity 3.5 pg/mL, cat# D8000C) and IL-10 (R&D, Human IL-10 QuantiGlo Chemiluminescent Sandwich ELISA, sensitivity 1.58 pg/mL, cat# Q1000).

Statistics

Statistical analyzes were performed using the Statistical Package for Social Science (SPSS) software, version 16.0 (SPSS Inc, Chicago, IL, USA). Normally distributed data are presented as group means and standard error of the mean (S.E.M.). Paired-samples t-test was used for evaluation of increasing or decreasing levels between two measuring points. Non-parametric statistics were used when Kolmogorov-Smirnov test and histograms demonstrated not normally distributed data, as shown for RBCT, coagulation-, fibrinolysis- and cytokine activation. Median levels are then presented. Wilcoxon Signed Rank Test was used as the non-parametric test describing increasing and decreasing levels between two measuring points. Differences were considered significant at P levels ≤ 0.05. No statistics were performed, and no figures are presented related to the patients that were initially externally fixated and later nailed due to the small number of patients.

Results

12 patients were included, 11 men and 1 woman, aged 27.6 ± 2.5 (range 18-44) years. The mechanisms of injury were car accident (10/12) and fall from heights (2/12). ISS was 31 ± 2.6, NISS was 34.3 ± 2.1, and AIS thorax was 3.7 ± 0.2. The time from injury to admission was 95 ± 11 (range 22 - 365) minutes. One patient had bilateral femoral shaft fractures. The fractures were classified according to AO; 3 A-fractures, 3 B-fractures and 7 C-type fractures. Two fractures were open (Gustilo Anderson 2 and 3A), and in 3 cases the fracture was openly reduced. All included patients had additional extremity- and thoracic injuries, 50% (6/12) abdominal, and 42% (5/12) had head injuries. The time from injury to femoral IMN was 544 ± 42 (range 330 – 830) minutes. The IMN operating time was 106 ± 7 (range 60 - 155)

minutes. Number of days in the intensive care unit (ICU) was 16 ± 4 (range 4 - 48). The postoperative course was prolonged by pneumonia (7/12), ALI (3/12), ARDS (3/12) and sepsis (3/12). All except one patient fulfilled the SIRS criteria. In the three patients who were initially externally fixated and secondarily nailed, the external fixation was performed 822 (range 284 – 1582) minutes after the injury, and the operating time was 45 (range 34 – 95) minutes. The time from injury to secondary IMN was between 6 and 10 days. In these patients mean ISS was 33 (range 29 – 34) and NISS 39 (range 34 – 43). 3/3 had pneumonia, 2/3 ALI, 1/3 ARDS, 2/3 sepsis and 3/3 had SIRS. Number of days in the intensive care unit (ICU) was 12 ± 3 (range 9 - 19).

None of the patients received blood product transfusions prior to hospital admission. Hemoglobin level at admission was 12.0 ± 0.7 and at skin incision 9.6 ± 0.4 g/100mL ($p = 0.015$). The body core temperature at admission was 36.4 ± 0.3. The variation of administrated blood products was wide, and the majority of transfusions were given between hospital admission and the first day post IMN (Table 2).

Coagulation and fibrinolysis

In all patients a marked activation of the coagulation- and fibrinolytic systems was seen at hospital admission. Arterial TAT levels decreased during the study period, whereas arterial sTF levels increased. Arterial PAI-1 levels were high at admission and increased further until 6 hours post procedure, the arterial t-PA activity levels were low at admission and stayed low during the study period, and the arterial t-PA antigen levels were high at admission and stayed elevated until decreasing from day one post IMN. No significant differences were demonstrated between arterial and mixed venous blood levels for TAT, sTF, t-PA activity or PAI-1 (data not shown).

Coagulation

Thrombin-antithrombin complexes (TAT)

The arterial TAT (Fig. 2a) levels were highest at hospital admission and decreased from admission to the third postoperative day. The decrease was significant from admission to skin incision ($p = 0.003$), and from admission to the third postoperative day ($p = 0.007$). No increase of arterial TAT plasma concentration related to the IMN procedure was observed.

Soluble tissue factor (sTF)

The arterial sTF (Fig. 2b) levels at admission were higher than normal levels and decreased from admission (A) to skin incision (B) ($p = 0.045$). A significant increase from nail insertion (C) to 72 hours (I) post nail insertion was found ($p = 0.011$). The highest sTF levels were present at the second and third postoperative day. No procedure-related effect could be detected.

Fibrinolysis

Tissue plasminogen activator (t-PA) activity and antigen

Low arterial t-PA activity levels were demonstrated during the whole observation period, without any significant procedure-related increase of t-PA activity (data not shown). The t-PA antigen level (Fig. 2c) was increased at hospital admission compared with normal levels in healthy adults and remained elevated before decreasing from 24 – 48 hours post procedure. No significant increases or decreases related to IMN were seen.

Plasminogen activator inhibitor-1 activity (PAI-1)

The arterial PAI-1 activity (Fig. 2d) levels at admission were elevated when compared to normal levels in healthy adults and increased further significantly from admission (A) to skin

incision (B) ($p = 0.001$) and from nail insertion (C) to six hours (F) ($p = 0.012$). From six (F) to 48 hours (H1) post nail insertion ($p = 0.005$) the PAI-1 activity levels decreased. Peak PAI-1 activity levels were demonstrated at skin incision (B) and at six hours post IMN (F). A procedure-related effect participating in the PAI-1 peak level response could not be ruled out.

Coagulation and fibrinolysis in patients that were primary external fixated and secondary nailed

The TAT, sTF and t-PA activity and antigen levels related to external fixation were similar to levels seen related to primary IMN. At secondary IMN a procedure-related increase of TAT, sTF and t-PA activity and antigen were present.

After external fixation only minor changes of the PAI-1 activity levels were present. The levels were lower than after primary IMN. Related to secondary IMN the PAI-1 response was almost absent.

Complement activation

Arterial TCC levels were studied in 6 patients. The TCC levels were slightly increased in most patients at admission and increased further (ns) from hospital admission (A) to the third postoperative day (I) (data not shown).

Cytokines

No significant differences were demonstrated between arterial and mixed venous blood levels for TNF-α, IL-6, IL-10, IL-1β, or IL-8.

TNF-α

The arterial TNF-α level remained steady and almost at normal levels from hospital admission to six hours after IMN (F) and then increased significantly to the third post IMN day (I) ($p = 0.036$) (Fig. 3a). The highest level was present the third post IMN day.

IL-6

Arterial IL-6 levels were elevated already at hospital admission, and for the whole study period the IL-6 levels exceeded 200 pg/mL. Peak levels were observed the third postoperative day (Fig. 3b). The increase from skin incision (B) to peak level at the third day post procedure was not significant ($p = 0.39$).

IL-10

Arterial IL-10 levels were elevated at hospital admission. A decrease from hospital admission to skin incision ($p = 0.009$) and a non-significant increase from skin incision to the first post IMN day ($p = 0.28$) was observed (Fig. 3c).

IL-1β and IL-8

IL-1β levels at all sampling locations were mostly below the lowest detectable level at 0.4 pg/mL (data not shown). IL-8 levels demonstrated no significant increases or decreases within the study group (data not shown).

Cytokine release in patients that were primary external fixated and secondary nailed

Peak TNF-α levels were observed at 30 minutes (D) after external fixation and after secondary IMN. Peak IL-6 level was present after external fixation and secondary IMN at the second postoperative day, but was below 200 pg/mL on the third postoperative day. After

external fixation IL-10 peaked 30 minutes post procedure, whereas related to secondary IMN no peaks of IL-10 were present.

Cardiopulmonary function

The $P_AO_2 - P_aO_2$ difference (Fig. 4a) demonstrated elevated and further increasing levels (ns) from skin incision (B) to the third postoperative day (I). Procedure-related increased levels could not be demonstrated. Significantly decreasing SaO_2 levels (Fig. 4b) from skin incision (B) to the third day post IMN (I) ($p = 0.000$) were observed. SvO_2 (Fig. 4c) decreased significantly from skin incision (B) to two hours after IMN (E) ($p = 0.046$).

CI (Fig. 5a) increased significantly from skin incision (B) to the third postoperative day (I) ($p = 0.000$). MAP demonstrated no significant changes during the study period (data not shown). The SVRI (Fig. 5b) levels were lower than normal at skin incision and the levels, with the exception of a minor procedure-related (ns) increase, and continued to decrease (ns) during the study period. The changes in the filling pressures in the right (CVP) and the left (PCWP) side of the heart were modest, but with levels in the upper range of normal levels (data not shown). Both CVP and PCWP demonstrated peak levels at 30 minutes post IMN (D), the increases, however, were not significant. The MPAP (Fig. 5c) levels were high at skin incision (B), had a minor transient procedure-related increase, and increased further from the first (G1) to the third postoperative day (I) (ns, $p = 0.07$). The PVRI levels (Fig. 5d) were high at skin incision (B) and increased (ns, $p = 0.057$) further from skin incision (B) until peaking at 2 hours after the nail was inserted (E).

Cardiopulmonary function in patients operated with external fixation and secondary nailing

$P_AO_2 - P_aO_2$ difference after external fixation demonstrated elevated and increasing levels from skin incision (B) to the first postoperative day (G1). Related to secondary nailing, the $P_AO_2 - P_aO_2$ difference was elevated at skin incision, and demonstrated decreasing levels during the study period. The SaO_2 levels after external fixation and secondary nailing were decreasing from skin incision (B) to the third postoperative day (I). Minor increases of PVRI, MPAP and PCWP values were seen between 30 minutes and two hours after secondary IMN.

Discussion

In this study on 12 polytraumatized patients with femoral shaft fractures we investigated the impact of IMN on cardiopulmonary function and inflammation- and coagulation/fibrinolytic responses. The main observation was that an additional effect of the primary IMN, a second hit, on the activation of coagulation and fibrinolysis could not be clearly identified. A transient procedure-related PVRI increase was present. This may be a result of intravasation of intramedullary content. Delayed peak levels of arterial TNF-α, IL-6 and IL-10 levels were observed. In three patients the femoral shaft fractures were initially externally fixated and secondarily nailed. After external fixation the activation of coagulation and fibrinolysis were similar to the activation related to primary IMN, the cytokine activation was lower and dropped earlier, and on the first and second day post procedure the pulmonary shunting was elevated when compared to primary IMN. After secondary nailing a procedure-related coagulation- and fibrinolysis activation, and with lower levels than after primary IMN, was present. The secondary nailing resulted in a procedure-related cytokine response, but this response was prior to- and with lower levels than after primary IMN. Neither secondary nor primary IMN had a negative impact on pulmonary shunting. As observed after primary IMN,

a procedure-related effect on PVRI, MPAP and PCWP was observed between 30 minutes and two hours post IMN.

In the present study systemic activation of the coagulation system, represented by arterial TAT level increase, was present at patient admission to the hospital. The high TAT levels were mainly related to the injury per se, and primary IMN did not result in additional TAT generation. Activation of fibrinolysis, measured as elevation of t-PA activity, was low. However, the t-PA antigen levels were high at hospital admission and stayed high before it dropped at 48 hours after IMN, which verify an increased fibrinolytic activity for much longer than the evaluation of t-PA activity alone suggests. The inhibition of the fibrinolysis, measured as augmented PAI-1 activity levels, was present at admission, and increased further. PAI-1 is produced upon stimulation by endothelial cells, platelets, fibroblasts and smooth muscle cells and functions as an acute phase reactant [20], and also binds circulating t-PA rapidly. Despite the prolonged increase in t-PA antigen levels, the functional effect of the PAI-1 increase (which is faster than other acute phase reactants like CRP and fibrinogen), combined with the nearly absent t-PA activity increase (neutralized by PAI-1) definitely represent a fibrinolytic shutdown and a prothrombotic state, which is often prevailing after orthopedic surgery or trauma. After external fixation, the activation of coagulation and fibrinolysis were similar to the activation observed after primary IMN. Related to secondary IMN, however, increased levels of both TAT and t-PA activities were measured.

Tissue factor (TF) is the most potent trigger of the coagulation system known[21]. sTF is normally not present at measurable levels in the circulation[22]. Marked peaks of plasma TF activity have been demonstrated during bone preparation in total hip replacement surgery[23], which indicates release of TF-rich material to the systemic circulation from traumatized

tissues, mainly the bone marrow which is a rich source of TF. This mechanism may also explain the sTF levels found in the present study; elevated arterial sTF blood levels were demonstrated at hospital admission (A), which decreased slightly until skin incision (B) and then increased significantly until the third postoperative day (I), indicating an enduring procoagulant state.

A significant cross-talk is present between coagulation, fibrinolysis, complement and inflammation[22, 24]. The complement cascade is activated by thrombin, and the complement split products stimulate TF synthesis in monocytes which in turn induces activation of the coagulation system[25]. The activation of the complement cascade occurs early (< 30 minutes) after trauma[26, 27]. In the present study, and in accordance with the literature, elevated and further increasing TCC levels (ns) were observed. However, no procedure-related increase of TCC levels was demonstrated, and the levels were lower than observed in the study by Fosse et al.[27] on severely injured patients. We have no good explanation for the difference between the present study and that of Fosse et al.[27], except that the ISS was lower (ISS 22, present study ISS 31) and the median time to hospital admission was 105 minutes (present study 95 minutes). The time wise relation between injury and peak TCC levels was not described by Fosse et al.[27].

Generation of proinflammatory cytokines has been demonstrated to be proportional to the extent of tissue injury and hypoxia[28-31]. TNF-α levels in our study were steady and slightly elevated until six hours post procedure (F) and were still increasing at day three post IMN. The still increasing levels at day three are not in agreement with other studies. Spielmann et al.[32] demonstrated the highest TNF-α level in trauma patients 12 hours post injury, with decreasing levels thereafter. Kobbe et al.[33] demonstrated no significant elevation of TNF-α.

Great variations in TNF-α levels (when measured) are observed in clinical studies, which can be explained by the short half-life of TNF-α[33].

The IL-6 levels in the present study were in general higher than 200 pg/mL, a level which is associated with SIRS[29]. In the one patient without SIRS, peak IL-6 level (at 6 hours post procedure) was 106 pg/mL. The elevated IL-6 levels in the present study were in agreement with other investigations demonstrating a post injury association between increased levels of IL-6, and high ISS and postoperative complications[28-31]. The IL-6 level peak in the present study occurred later than peak levels observed in other studies[8, 9, 28, 30, 34] in which IL-6 peaked 4 - 24 hours post injury or post procedure and persisted for 3 - 10 days[30, 34]. Orthopedic surgery has in particular been associated with local release of IL-6[35]. Pape et al.[9] investigated patients with femoral shaft fractures and found venous IL-6 peak levels 24 hours after IMN. When the femoral fracture was initially stabilized with external fixation and secondarily converted to an intramedullary implant, they did not, however, observe a surgery-related IL-6 increase[9]. These results are inconsistent with the results in the present study demonstrating arterial IL-6 peak level at the second postoperative day after primary IMN and also a procedure-related IL-6 response after secondary IMN. The IL-6 levels after secondary nailing in our study were significantly lower than the levels after primary IMN. The results from the study of Pape et al.[9] were also inconsistent with the results from the study of Morley et al.[36] in which blood samples from the femoral canal before and after reaming of the canal showed very high levels of IL-6 after intramedullary reaming (median 3947 at opening and 15903 pg/mL after reaming). These very high levels indicated a significant local inflammatory reaction following the dual trauma (fracture and IMN). Due to intravasation and pulmonal sequestering of bone marrow content at intramedullary pressure increase during femoral canal reaming, an additional pulmonary IL-6 activation has been suggested in an

experimental porcine study[37]. Levels of IL-6 in both mixed venous and arterial blood were analyzed in the present study, but the results did not confirm the suggestion.

No IL-1β or IL-8 response was observed in the present study. This is consistent with other studies[33, 37, 38].

For the evaluation of the anti-inflammatory response IL-10 was studied. In the present study, related to primary IMN, IL-10 levels were elevated during the study period, and peak IL-10 level was observed at the first day post primary IMN. This IL-10 response was delayed compared to other studies demonstrating an IL-10 response after 1 - 6 hours post injury[33, 39].

In the literature, there is no uniform understanding of the effect of IMN on hemodynamics and pulmonary function. Early operative fracture treatment, especially of large long bones (femur), has empirically been associated with reduction in the occurrence of pulmonary failure (ARDS)[40-42]. However, some data also indicate that the early internal stabilization of these fracturesin itself can have negative impacts on pulmonary function. The total effect of IMN on morbidity seems, however, to be beneficial.

The reported incidences of ALI and ARDS in severely injured patients with femoral shaft fractures are not uniform[42-44]; a higher incidence is suggested if the fractures are accompanied by thoracic injuries[43]. In the present study 6/12 patients had ALI or ARDS (3/12 ALI and 3/12 ARDS). The pathophysiology of the early ALI after trauma is fluid leakage and pulmonary edema combined with inflammatory cell infiltration of pulmonary tissue. In addition, a hypoxemic vasoconstriction and capillary microthrombosis creates a

ventilation-perfusion mismatch, clinically manifested as hypoxemia. The P_AO_2 - P_aO_2 difference increased in the severely injured patients in this study as a surrogate indicator for increased pulmonary shunting. Pulmonary vascular changes may cause an increased pulmonary vascular resistance and increase the MPAP[45]. In the present study MPAP was elevated already at skin incision, and demonstrated a minor procedure-related increase, before continuously increasing towards the end of the study period. Simultaneously, a procedure–related PVRI level increase was observed. In previous human studies increased MPAP, PVR and CVP plus decreased PaO_2 have been associated with pulmonary embolism[46]. These associations, however, have to be interpreted carefully as pressure increases in previous normal, easily distendable, pulmonary blood vessels may retain undetectable until at least 30% of the vessels are occluded[47]. In pre-injured lungs the effect of compensatory dilatation will not occur equivalently, and the flow in the still open pulmonary vessels will increase and create significantly elevated PVR levels. The filling pressures of the heart, CVP and PCWP, are influenced by treatment, quantum of intravenous fluid/blood administration and the titration of ventilator volumes, pressures and rates. As strict control of these parameters is impossible in a clinical setting, CVP and PCWP are not suitable for the evaluation of impact of surgical interventions on cardiac function.

Polytraumatized patients usually consist of a heterogeneous group of patients, and firm conclusions from clinical studies are difficult to draw. Differences in the extent of injury, time from injury to hospital admission, time from admission to operation, extent of further operations and amount of blood product transfusions induce a wide range of variance. The included patients in the present study were extensively examined, which also made the patient inclusion and follow-up challenging. The present patient material consisted of patients with high ISS, femoral shaft fractures equally treated, and all registrations, calculations and analyzes were strictly related to IMN. However, the included patients were relatively few.

We did not differentiate and relate the observations and calculations to specific organ system injuries. For such differentiation, larger studies and multi-centre trials are needed.

Conclusions

In the severely injured patients with femoral shaft fractures operated with primary IMN we observed a substantial response related to the initial trauma. We could not demonstrate any major additional IMN-related impact on theinflammation or the cardiopulmonary function parameters. A transient cardiopulmonary response related to IMN, however, was demonstrated. Delayed arterial TNF-α, IL-6 and IL10 peak levels were observed, and it could be questioned whether these were related to the procedure. The inflammation-associated complication rate was high.

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Figure legends

Figure 1. Flow chart of patients admitted with femoral shaft fracture and included in the study.

Figure 2. The figure shows the arterial TAT (2a), sTF (2b), t-PA antigen (2c) and PAI-1 activity (2d) (median and 25/75 percentiles) at admission (A), skin incision (B), after nail insertion (C) and 30 minutes (D), two (E) and six (F) hours, the first (G1), second (H1) and third (I) day after the nail insertion in the patients primarily nailed.

Figure 3. The figure shows the arterial TNF-α (3a), IL-6 (3b) and IL-10 (3c) (median and 25/75 percentiles) at admission (A), skin incision (B), after nail insertion (C) and 30 minutes (D), two (E) and six (F) hours, the first (G1), second (H1) and third (I) day after the nail insertion in the patients primarily nailed.

Figure 4. The figure shows $P_AO_2 - P_aO_2$ difference (Fig. 4a), SaO_2 (Fig. 4b) and SvO_2 (Fig. 4c) (mean ± S.E.M.) at skin incision (B), after nail insertion (C) and 30 minutes (D), two (E) and six (F) hours, the first (G1 and G2), second (H1 and H2) and third (I) day after nail insertion in the patients primary nailed.

Figure 5. The figure shows the time course (mean ± S. E. M.) for CI (Fig. 5a), SVRI (Fig. 5b), MPAP (Fig. 5c) and PVRI (Fig. 5d) at admission (A), skin incision (B), after nail insertion (C) and 30 minutes (D), two (E) and six (F) hours, the first- (G1 and G2), second- (H1 and H2) and third (I) day after primary IMN.

Figure 1

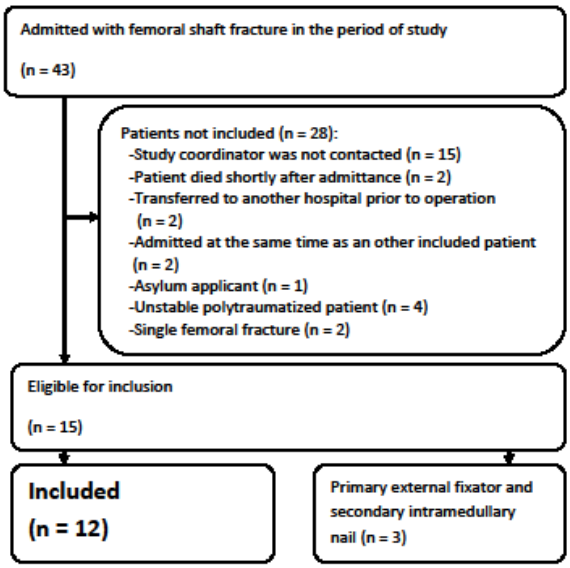

Admitted with femoral shaft fracture in the period of study
(n = 43)
Patients not included (n = 28):
-Study coordinator was not contacted (n = 15)
-Patient died shortly after admittance (n = 2)
-Transferred to another hospital prior to operation (n = 2)
-Admitted at the same time as an other included patient (n = 2)
-Asylum applicant (n = 1)
-Unstable polytraumatized patient (n = 4)
-Single femoral fracture (n = 2)
Eligible for inclusion
(n = 15)
Included
(n = 12)
Primary external fixator and secondary intramedullary nail (n = 3)

Figure 2

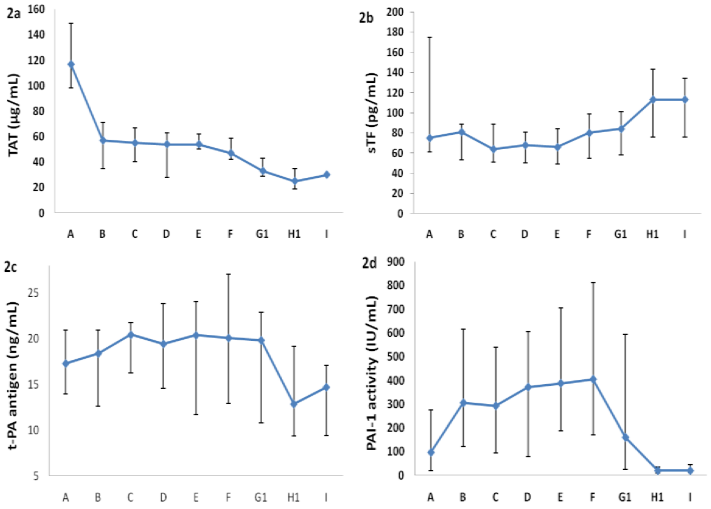

2a
TAT (µg/mL)
160
140
120
100
80
60
40
20
0
A B C D E F G1 H1 I
2b
sTF (pg/mL)
200
180
160
140
120
100
80
60
40
20
0
A B C D E F G1 H1 I
2c
t-PA antigen (ng/mL)
25
20
15
10
5
A B C D E F G1 H1 I
2d
PAI-1 activity (IU/mL)
900
800
700
600
500
400
300
200
100
0
A B C D E F G1 H1 I

Figure 3

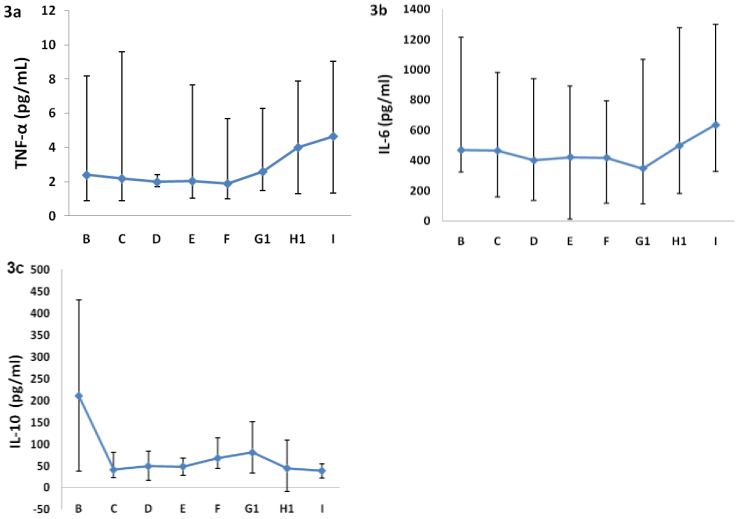

3a
TNF-α (pg/mL)
12
10
8
6
4
2
0
B C D E F G1 H1 I
3b
IL-6 (pg/ml)
1400
1200
1000
800
600
400
200
0
B C D E F G1 H1 I
3c
IL-10 (pg/ml)
500
450
400
350
300
250
200
150
100
50
0
-50
B C D E F G1 H1 I

Figure 4

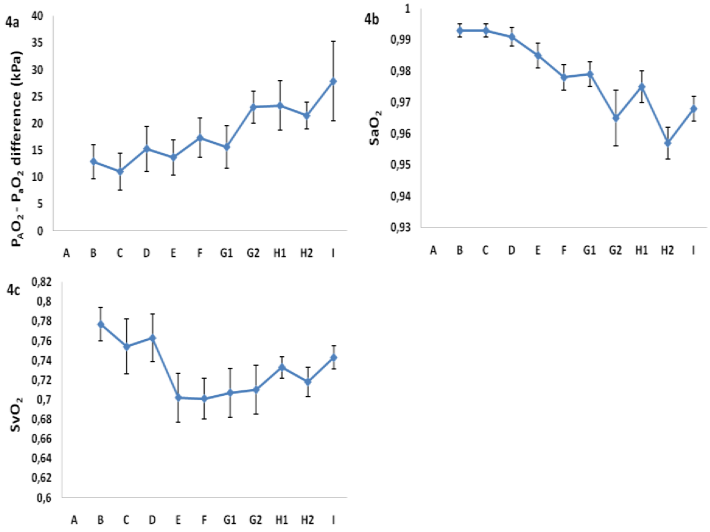
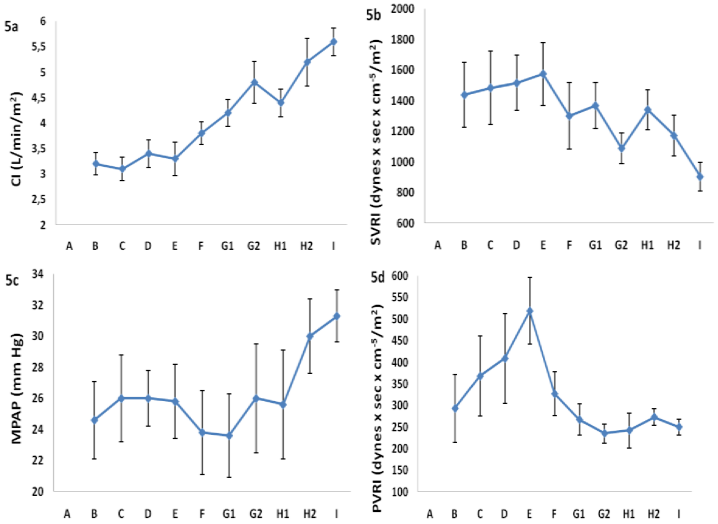

4a
$P_aO_2 - P_aO_2$ difference (kPa)
40
35
30
25
20
15
10
5
0
A B C D E F G1 G2 H1 H2 I
4b
SaO_2
1
0,99
0,98
0,97
0,96
0,95
0,94
0,93
A B C D E F G1 G2 H1 H2 I
4c
SvO_2
0,82
0,8
0,78
0,76
0,74
0,72
0,7
0,68
0,66
0,64
0,62
0,6
A B C D E F G1 G2 H1 H2 I

Figure 5


5a
CI (L/min/m²)
6
5,5
5
4,5
4
3,5
3
2,5
2
A B C D E F G1 G2 H1 H2 I
5b
SVRI (dynes x sec x cm⁻⁵/m²)
2000
1800
1600
1400
1200
1000
800
600
A B C D E F G1 G2 H1 H2 I
5c
MPAP (mm Hg)
34
32
30
28
26
24
22
20
A B C D E F G1 G2 H1 H2 I
5d
PVRI (dynes x sec x cm⁻⁵/m²)
600
550
500
450
400
350
300
250
200
150
100
A B C D E F G1 G2 H1 H2 I

Tables

Table 1. Time schedule for analysis, registrations and calculations

Time	Surgical procedure	Blood sample analyses	Cardiopulmonary registrations and calculations
A	Hospital admission	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	
B	Skin incision	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
C	After nail insertion	Blood gases, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
D	30 minutes after nail insertion	Blood gases, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
E	2 hours after nail insertion	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
F	6 hours after nail insertion	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
G1	1. postoperative day at 0800	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
G2	1. postoperative day at 1800	Blood gases	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
H1	2. postoperative day at 0800	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
H2	2. postoperative day at 1800	Blood gases	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference
I	3. postoperative day at 0800	Blood gases, Hb, cytokines, coagulation, fibrinolysis, complement	CI, SVRI, PVRI, HR, MAP, CVP, MPAP, PCWP, SvO_2, P_AO_2-P_aO_2 difference

Table 1. The table shows the time schedule for blood sampling and cardiopulmonary registrations and calculations at hospital admission (A), skin incision (B), after insertion of the nail (C), and at 30 minutes (D), two (E) and six (F) hours after nailing, and at 0800 (G1) and 1800 hour (G2) the first-, 0800 (H1) and 1800 hour (H2) the second- and at 0800 (I) hour the third postoperative day.

Table. 2. Blood product transfusions

Time	RBCT	TT	PT
Admission - IMN (A-B)	900 (0-4800)	0 (0-500)	0 (0-400)
IMN - 6 hours post IMN (B-F)	750 (0-2100)	0 (0-500)	0 (0-400)
Admission - 1. postoperative day (A-G1)	2250 (0-6900)	0 (0-1000)	0 (0-1400)
1. - 2. postoperative day (G1 - H1)	600 (0-1500)	0 (0)	0 (0-400)
2. - 3. postoperative day (H1 - I)	300 (0-600)	0 (0-500)	0 (0-400)
TOTAL	**3000 (0-8100)**	**0 (0-1500)**	**200 (0-1400)**

Table 2. The table gives a summary of administrated red blood cell transfusion (RBCT), thrombocyte transfusion (TT), blood plasma transfusion (PT) in milliliter (median and range) from hospital admission to intramedullary nailing (IMN), from IMN to 6 hours after IMN, from hospital admission to the first postoperative day, from the first to the second postoperative day, from the second to the third postoperative day and total transfusions in the patients primary nailed.